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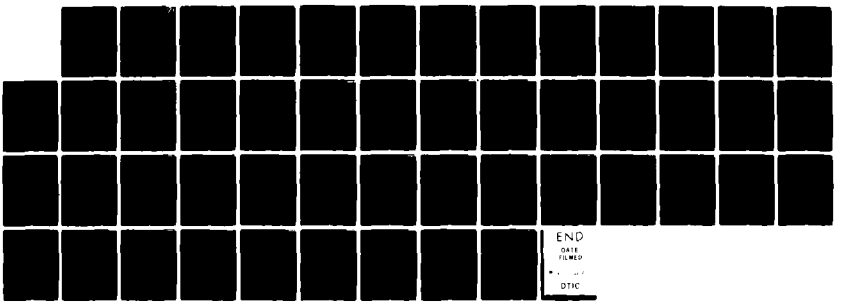

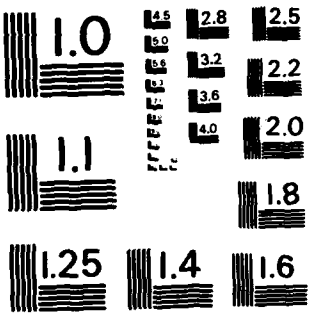

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The Biophysical Properties of Stroma Free Hemoglobin and Whole Blood Mixtures

Final Report

L. C. Cerny

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This is a preliminary investigation to establish and to develop the biophysical methodology for the evaluation of the use of stroma-free solutions as blood substitutes.

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Stroma-free hemoglobin solutions are promising plasma substitutes. They have satisfactory oxygen-carrying capacity, do not have any adverse effects on the renal function and offer some rheological advantages. An ideal plasma substitute should exert a sufficient colloidal osmotic pressure to maintain the circulating volume, should not increase the blood viscosity, especially at near stagnant conditions, should aid in the improvement of tissue perfusion and should not interact with the erythrocyte membrane or plasma proteins to cause aggregation. Stroma-free hemoglobin (SFH) meets these criteria. However, improvements are always taking place such as the amidination and pyridoxilation of the hemoglobin to increase the oxygen binding capacity, lipid encapsulation of the hemoglobin to prolong its in vivo retention, and the addition of suitable polymers to promote consistent rheological behavior. Because of the vast amount of work and interest in SFH and its modifications, it is extremely important to establish a data base through satisfactory model experiments as a means of recognizing and evaluating quantitatively the effects of any changes that may be made.

Rheological Properties:

The viscosity of the stroma-free hemoglobin (SFH) was determined in a Cannon-Ubbelohde Semi Micro Dilution Type

viscometer at 37°C. In all cases the kinetic energy correction factor was small enough to be neglected. The diluent was physiological saline at pH 7.4. The concentrations of these solutions were in the range from 2.5 to 30 gm/100 ml of Hgb. These data are shown graphically in (Fig.1) along with a similar set of measurements on albumin solutions. It should be noted that below the 10 gm/100 ml level, there is very little difference between the relative viscosities (η_{rel}) of the two solutions. The viscosity of the SFH solutions could be quantitatively represented by the generalized Mooney[1] equation,

Fig.1

$$\ln \eta_{rel} = \frac{[\eta]c}{1 - (k/v)\,[\eta]c} \qquad (1)$$

In equation 1, $[\eta]$ is the intrinsic viscosity in dl/g, c, the concentration (g/dl) and k/v is a parameter which takes into account the molecular asymmetry and "crowding". From our data, $[\eta]$ was found to be 0.045 dl/g and k/v was 0.38. Using these values, it was possible to represent the experimental data to better than 5% deviation.

Simha[2] showed that the intrinsic viscosity of disc-like particle could be represented by

$$[\eta] = \frac{16f}{15 \tan^{-1} f} \qquad (2)$$

where f was the axial ratio. If one uses the values given by Tanford[3], f can be taken as 2.1 to 3.4, yielding an intrinsic viscosity between 0.0347 and 0.0493 dl/g which agrees well with our experimentally determined value.

To examine the effects of mixtures of SFH and plasma protein, viscosity measurements were made using two different SFH starting concentrations; one of 14.5 gm/100 ml and the other 7.25 gm/100 ml. These data are shown in (Fig.2) where the specific viscosity, η_{sp}, divided by the concentration is graphed versus the weight fraction of plasma protein. The specific viscosity is used because it represents the contribution to the viscosity due to the amount of polymer (SFH and/or protein) present above that of the solvent. The figure indicates a linear relationship. For comparison, mixtures of SFH (7.25 gm/100 ml) and albumin (6.5 gm/100 ml) are also graphed in (Fig.2). Fig.2 Fig.2

The effect of shear rate on viscosity of SFH has not been definitely resolved. At high concentrations (i.e. above 15%), shear thinning may be a controlling factor in the microcirculation. Some of these measurements are shown in (Fig.3A) and (Fig.3B) where the viscosity (centipoise) is graphed versus rate of shear (sec^{-1}). These measurements were made in a multi-bulb Ubbelohde viscometer and in a cone plate viscometer. Fig.3A Fig.3B

The non-Newtonian flow behavior of several mixtures of whole blood and SFH was examined. In(Fig.4), the viscosity at four different hematocrits is graphed versus the shear rate with a 6.1% SFH solution as the diluent. Below 30% Hct, the flow is Newtonian and independent of the shear. The effect of hemodilution is quite apparent and has a marked effect on the viscosity reduction. A similar experiment is illustrated in(Fig.5). In this investigation, the diluent is 14.5% SFH solution. The non-Newtonian flow region even extends to the 17.7% Hct mixture. This is in part due to the non-Newtonian behavior of this concentration of SFH which is indicated in(Fig.3). The hemodilution effect is not as pronounced as with the 6.1% SFH solution as illustrated with the samples of 53 and 42.4% Hct.

Fig.4

Fig.5

Fig.3

In addition to the flow properties, the rate of oxygen transport is of equal importance for cardiovascular and respiratory activity. As an indication of this property, it is normal to express the ratio of the hematocrit to the viscosity (Hct/η) as a function of the hematocrit (Hct) at a fixed shear rate[4]. This is shown in(Fig.6) for whole blood diluted with 6.1% SFH solution at the shear rates of 46 sec^{-1} and 23 sec^{-1}. These shear rates were chosen because they illustrated the greatest effect of this activity. The graph indicates that

Fig.6

a maximum does exist at a hematocrit of about 25%. Similar data are shown in (Fig.7). However in this graph, the diluent was 14.5% SFH solution. It is important to observe that the overall effect of this activity is reduced, there is no maximum in the curve and the effect is almost constant and independent of hematocrit. This indicates that the oxygen transport is apparently not directly increased with increasing SFH concentration. This is a complex relationship and intimately involved with the flow patterns in(Fig.4 and 5).

Fig.7

Fig.4
Fig.5

Osmotic Pressure:

The measurement of the colloidal osmotic pressure was determined with a modified Zweifach micro-osmometer. A constant temperature bath was used to control the temperature to better than $\pm 0.5^{\circ}C$. The concentration range extended to about 14 gm/100 ml. It was shown that the data could be represented by

$$\left(\frac{\pi}{c}\right)^{1/2} = \left(\frac{\pi}{c}\right)_o^{1/2} \left[1 + \frac{\Gamma_2 c}{2}\right] \qquad (3)$$

In this equation, π represents the osmotic pressure at concentration C, and Γ_2 is the second virial coefficient which is a measure of the polymer-solvent interactions. A graph of $\left(\frac{\pi}{c}\right)^{1/2}$ vs. C results in a straight line. From the intercept, $\left(\frac{\pi}{c}\right)_o^{1/2}$ one obtains the number average molecular weight, Mn.

$$\left(\frac{\pi}{c}\right)_o = \frac{RT}{Mn} \qquad (4)$$

From the slope, one evaluates Γ_2,

$$\Gamma_2 = \frac{2\ (\text{slope})}{\left(\frac{\pi}{c}\right)_o^{1/2}} \qquad (5)$$

Equation (3) is used for the osmotic pressure rather than a plot of $\frac{\pi}{c}$ vs. C, because it eliminates the curvature which may lead to erroneous extrapolation procedures.

The osmotic pressure data are summarized in (Table 1) Table for three different temperature. Also included are the osmotic pressure data for other commonly used plasma expanders, hydroethyl starch (HES), dextran, polyvinylpyrrolidone (PVP) and albumin. The values of $\Gamma_2/[\eta]$ are presented because they should remain constant for a particular polymer series.

It is interesting to note the magnitude of the Γ_2 values for SFH in comparison to the series of other plasma expanders. The relative size indicates that the SFH solutions are approaching the ideal polymer solution, $\Gamma_2=0$.

Some data of mixtures with SFH and plasma proteins are shown in(Fig.8). In this graph the osmotic press/concentration (π/c) is graphed versus the weight fraction of plasma. Two SFH concentrations are presented, 7.25 gm/100 ml and 14.5 gm/100 ml. For comparison, some data with 7.0 gm/100 ml SFH and 6.5 gm/100 ml albumin mixtures are also graphed.

The osmotic pressure of mixtures of whole blood at different concentrations of SFH also present an interesting situation. These data are shown in (Fig.9). In this Fig. figure, the osmotic pressure in cm. of H_2O is graphed against the volume percent of SFH in the mixture. These determinations were made at four SFH concentrations, 3.5%, 7.0%, 9.5% and 15.2%. The values of the blood hematocrits are given in parenthesis on the graph. It is interesting to observe the maxima that occurs at the concentrations of 7.0% and greater at about 75 volume % of SFH. This would suggest that during infusion with SFH solutions the vessels in the circulatory system are in a hyper osmotic state relative to whole blood. This may have certain advantages in the treatment of shock.

Erythrocyte Sedimentation Rate:

The erythrocyte sedimentation rate (ESR) studies were made in the automatic sedimentimeter[5]. This unit not only follows the settling process but it also electronically generates the first derivative of the curve, that is, the true rate of settling as a function of time. This is a new concept in ESR and has the potential of becoming a diagnostic indicator. A typical set of data are shown in (Fig.10) where both the Fi settling curves and the first derivatives are taken directly from the sedimentimeter. A summary of data is presented in

(Table 2). It is our contention that the maximum velocity, Table2
V_{max} (cm/hr), is the most meaningful single parameter to document the ESR.

A careful examination of(Table 2)indicates some Table 2
interesting results. First, it appears that a total replacement of the plasma with either a 7% SFH solution or a 6.5% albumin solution eliminates all settling. This could be interpreted to mean that the forces which aggregate the red cells causing the settling, have been eliminated. To compare a commonly used plasma expander with SFH two samples of hydroxyethyl were used (High MW=450,000; Low MW=264,000). Both samples over a wide range of concentration greatly increase the ESR.

The interesting feature relates to the whole blood case with different volume ratios of 6% SFH solution. These ratios are typical of those used in infusion procedures. It is observed that there is only a small change in the ESR over this clinically significant range of whole blood-SFH mixtures.

Malonamide Kinetics and Osmotic Fragility:

We have shown that the technique of hemolytic malonamide kinetics offers a means of examining the properties of the red cell membrane[6]. This method is particularly convenient and accurate for dilute solutions of SFH, that is, a 7% solution. The optics of the spectrophotometer place a limit on this

technique. In this investigation, we were concerned with the effect of overnite incubation of whole blood and 7% SFH mixtures. Previously we indicated that the kinetics could be followed by the simple mathematical expression

$$\text{Percent Hemolysis} = \frac{1}{1 + \text{EXP}\ [\beta(t-t_{50})]} \qquad (6)$$

In equation (6), t represents the time, t_{50} is the half life of the reaction and β is a parameter measuring the breadth of the red cell kinetic activity. In (Fig.11), we have a sample of whole blood as the control. In (Fig.12) data are presented using 75% by volume of 7% SFH solution whole blood mixtures. Fig.11 Fig.12

The values of the t_{50} are given on each figure. There does not appear to be any significant change in the hemolytic kinetics caused by the presence of the SFH solution. The data are summarized in (Table 3). Table 3

The kinetic hemolysis described above measures the ability of the red cell membrane to withstand a slowly increasing osmotic presure (30 minute time period). It also provides information on whether or not the SFH present interferes with the transport of relatively small molecules (i.e. malonamide) across the erythrocyte membrane.

In order to measure the effect of the presence of SFH

on the red cells response to rapidly increasing osmotic pressure (1 to 2 minute time period) data were taken using a Kalmedic Fragilograph[7]. This instrument provides simultaneously cummulative and derivative hemolysis data as a function of decreasing extracellular sodium chloride concentration. These data give the mean fragility of the red cell population. The skewness of the derivative curve immediately indicates when a particular segment of the population is being preferentially attacked by a hemolytic agent. All measurements were made at 37°C using a sodium chloride-sodium veranol buffer at pH-7.4. Three experimental conditions were used. In the first instance the fragility of a mixture of 7% SFH with whole blood (50/50 by volume) was compared with a sample of the whole blood. Both of the samples were incubated at 4°C and the measurements repeated after 4 hours. Typical data can be seen in (Fig.13) and (Fig.14). The presence of the SFH resulted in a slight (7 to 8%) increase in fragility. The extra cellular saline concentration at which half the red cell population had hemolyzed, C_{50}, is listed on the graph for comparison.

Fig.13
Fig.14

The second series of experiments repeated the above protocol except that the samples were kept at room temperature (22°C) rather than 4°C. Representative curves are shown in (Figs.15 and 16). As can be seen from the C_{50} values listed

Fig.15
Fig.16

on the figure, the presence of SFH does not alter the hemolysis behavior of the erythrocytes.

Neither of the above procedures showed a significant difference in the hemolysis in the presence or absence of SFH. Similar experiments identical to those above except that the hemoglobin solution was not mixed with the whole blood until immediately before the fragility determination also showed no difference among the various samples.

The last data set compared initial fragility values with those from samples incubated for 24 hours at 37°C. An average data set is shown in (Figs. 17 and 18). It is most easily seen from (Fig.18) that initially there is no discernable difference between the whole blood and Hgb whole blood mixture. After incubating for 24 hours both samples showed an increased fragility. It should also be noted that both derivative curves are broadened but remain very symmetrical. This indicates that no given part of the population (say, only older erythrocytes) are being affected more by the treatment of the SFH. Careful attention should be given to the relative positions of the derivative curves. The SFH mixture seems to offer significant protection to the red cells during the incubation. The addition of SFH does not prevent the increase in fragility noted for the whole blood sample, but it does reduce this increased fragility. This is best seen quantitatively

Fig.1
Fig.1

by comparing the C_{50} values listed on(Fig.17). Both of Fig.17
the above experimental procedures indicate that the addition of SFH does not increase the susceptibility of the red cell to osmotic fragility and may offer a slight but significant degree of protection.

Some New Directions:

Since one of the major concerns with the clinical use of SFH is its relatively short dwell time, two approaches to this problem are currently being investigated. The first deals with mixtures of hydroxyethyl starch (HES) and SFH and the second approach involves the formation of an SFH polymer complex.

In (Fig.19) the oncotic pressures of low molecular Fig.19
weight HES (Mw=264,000), high molecular weight HES (Mw=450,000) and SFH are compared. There are differences but they are not of major significance. However, if the relative viscosities are examined, as shown in (Fig.20), notable differences are Fig.20
readily apparent. It is possible to lower the viscosity of the HES by the addition of SFH. This is illustrated in (Fig.21) Fig.21
for both of the HESes. The osmotic pressure was determined on the mixtures for the high molecular weight HES with SFH. The effect is not as dramatic but this is related to the similarities in the osmotic pressures of the pure materials.

However, detailed *in vivo* testing will be necessary to substantiate the usefulness of the mixtures (Fig.22). Fig.22

Current work in our laboratory has indicated that it is possible to react Hgb with water-soluble polymers[8]. Two of these compounds have been synthesized. The first is shown on (Fig.23), between HES and Hgb. The characterization of Fig.23 this polymer indicates a number average molecular weight of about 275,000. This indicates that about 3 Hgb molecules have reacted with each HES molecule of number average molecular weight 65,200. However at a 3 gm/100ml concentration level, the relative viscosity is approximately 2.1 times the viscosity of SFH and therefore may present a problem. If a fractionated HES sample is used with a lower molecular weight, there will be better control of both the viscous and oncotic properties.

Another approach to synthesizing a water soluble polymer with oxygen carrying properties has been to use a Tetronic polyol as a starting material. These compounds are four-pronged polymers of ethylene and propylene oxides. They are shown in (Fig.24). It is possible to convert the alcohol Fig.24 groups to a tetra aldehyde under very mild conditions[9]. By regulating the reaction conditions, it appears that it is possible to obtain nono-, di-, tri-and tetra-substitution. Our current interest concerns the separation of these mixtures.

Conclusions:

The preliminary results of this study indicate that the biophysical characteristics of SFH make it an extremely suitable blood substitute. One of the key factors in this conclusion is its availability from out-dated whole blood. At the concentration that is currently being considered as an infusing solution, 7%, it is a Newtonian fluid having a viscosity very similar to plasma. At this concentration, SFH also has very favorable hemo-dilution properties. This is a feature which is a critical factor for any plasma substitute. However, the added advantage here is the potential oxygen transport ability which most expanders lack. There also appears to be little interaction between the plasma proteins and SFH in the concentration range between 7 and 14% as indicated from viscosity measurements. However, at concentrations exceeding 10% SFH solutions exhibit a slight non-Newtonian flow pattern that may present some complications in the microcirculation and distract from its advantage as a hemo-diluent.

The osmotic behavior of SFH solutions indicate that it approaches an ideal polymer-solvent system as illustrated by the small value of the second virial coefficient, especially when compared to other plasma substitutes including dextran, hydroxyethyl starch and polyvinyl pyrrolidone. In mixtures with whole blood at a series of hematocrits, SFH solutions at

concentration between 7 and 15.2% all appear to be hyper osmotic. This biophysical property could certainly have significant ramifications in cases of severe shock.

Another notable feature of SFH solutions is its ability to maintain the integrity of whole blood and not to initiate aggregation of the erythrocytes. A common technique which illustrates this phenomena is the ESR. With some plasma expanders, such as dextran and polyvinyl pyrrolidone, there is a noticeable increased ESR. Our studies with HES, both the high and low molecular weight types, also indicate an increased ESR. The investigations of the ESR in this study with SFH, either alone with whole blood or in combination with the low molecular weight HES, indicate the potential usefulness of SFH in maintaining stability in these systems. The fact that SFH does not promote erythrocyte aggregation is another advantage that it may have as an infusion fluid for the treatment of shock.

In the presence of SFH solutions, the transport properties of the red cell membrane are apparently not effectively altered. This has been illustrated in the hemolytic malonamide kinetic studies. The negligible changes in the half life values for this kinetic process over a twenty-four hour period substantiate this fact. To further explore the erythrocyte membrane properties similar studies of the osmotic fragility were performed.

Although the results are preliminary, it should be stressed that SFH solutions may offer protection against hemolysis when used as a transfusion solution.

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Fig.16 Derivative Hemolysis Curves at 22°C.

Fig.17 Cumulative Hemolysis Curves at 37°C.

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Table 1

The Osmotic Pressure and Intrinsic Viscosity of Stroma-Free Hemoglobin and Some Plasma Substitutes

Substance	M_n	Γ_2 $(\frac{ml}{gm})$	$[\eta]$ $(\frac{dl}{gm})$	$\Gamma_2/[\eta]$
		Temperature 37°c		
SFH	69,200	3.59	0.0385	93.2
Albumin	81,000	7.01	0.0380	185.0
		Temperature 30°c		
SFH	67,300	3.49	0.0350	99.7
Dextran-Rheomacrodex	33,400	12.4	0.175	70.9
Dextran-Macrodex	49,100	20.2	0.275	73.3
PVP-Plasdone	23,700	8.68	0.202	43.0
Hydroxyethyl-Starch	81,500	21.2	0.181	117
		Temperature 20°c		
SFH	66,800	3.21	0.0320	100.3

Table 2

The Effects of Replacement Fluids on the Erythrocyte Sedimentation Rate

	System	Hct	Vmax ($\frac{cm}{hr}$)
A	1) Whole Blood	41	3.67
	2) Washed cells in 7% SFH	41	no settli
B	1) Whole Blood	36	7.98
	2) Washed cells in 50% plasma+50% 7% SFH	36	1.77
	3) Washed cells in 50% plasma+50% 0.9% Saline	36	1.01
C	1) Whole Blood	42	11.08
	2) Washed cells in 66% plasma-34% 10% SFH	42	4.62
D	1) Whole Blood	42.5	4.69
	2) Washed cells in 6.5% Albumin	42.5	no settli
E	Washed Cells in		
	1) 1% High MW HES	38	1.01
	2) 2% High MW HES	38	6.46
	3) 3% High MW HES	38	18.37
	4) 4% High MW HES	38	26.98
F	Washed Cells in		
	1) 2% Low MW HES	37	0.63
	2) 4% Low MW HES	41	6.21
	3) 6% Low MW HES	38	10.39
G	Washed Cells in		
	1) 6% Low MW HES+0% SFH	36	11.15
	2) 6% Low MW HES+0.606% SFH	36.5	5.32
	3) 6% Low MW HES+0.303% SFH	37	8.11
H	Whole Blood	38	6.78
	1) 6% SFH-Whole Blood (1v:6v)	31.5	5.95
	2) 6% SFH-Whole Blood (1v:7v)	32	5.95
	3) 6% SFH-Whole Blood (1v:9v)	34	5.95

Table 3
Malonamide Induced Hemolytic Kinetics
Effect of Stroma Free Hemoglobin Concentration
and Volume Percent Whole Blood

SFH Conc.		Control Blood Sample	Overnight	75% Control 25% SFH	Overnight	50% Control 50% SFH	Overnight	25% Control 75% SFH	Overnight
3.17%	β	0.591	0.485	0.437	0.457	0.539	0.403	0.475	0.423
	t50	15.5	17.0	13.9	14.8	12.9	15.4	12.1	12.9
7.00%	β	0.271	0.276	0.215	0.222	0.256	0.232	0.239	0.261
	t50	20.7	18.3	16.6	14.9	17.4	15.9	17.7	18.2
	β	0.407	0.359	0.259	0.395	0.328	0.383	0.384	0.386
	t50	15.8	14.8	15.2	16.3	14.7	16.2	16.3	16.7
9.5%	β	0.358	0.333	0.451	0.443	0.336	0.284	———	0.195
	t50	19.4	21.1	17.2	16.7	16.9	18.2	———	19.5

$$\text{Percent Hemolysis} = \frac{1}{1+\text{Exp}\ [\beta(t-t50)]}$$

β in min^{-1}

t50 in min

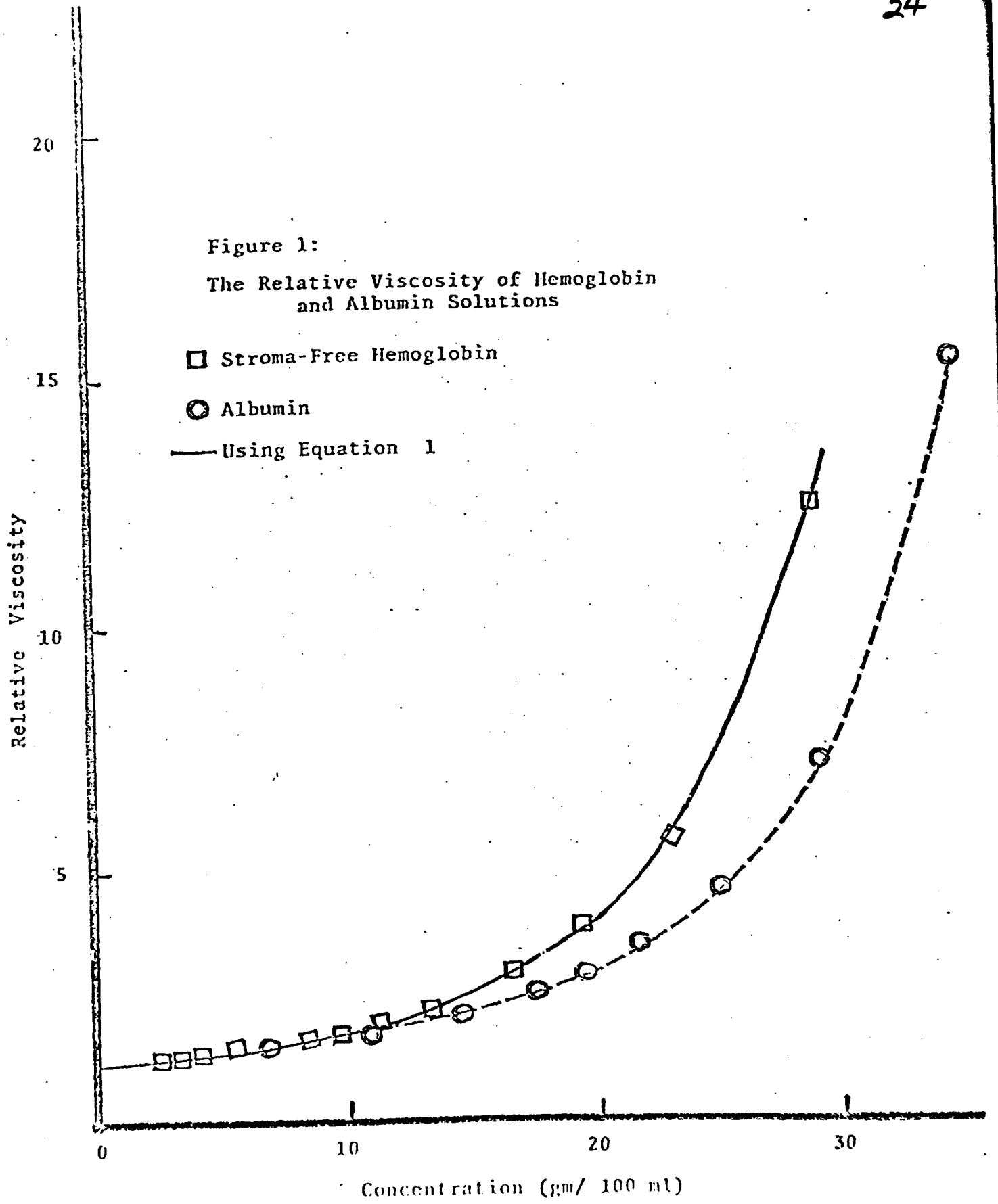
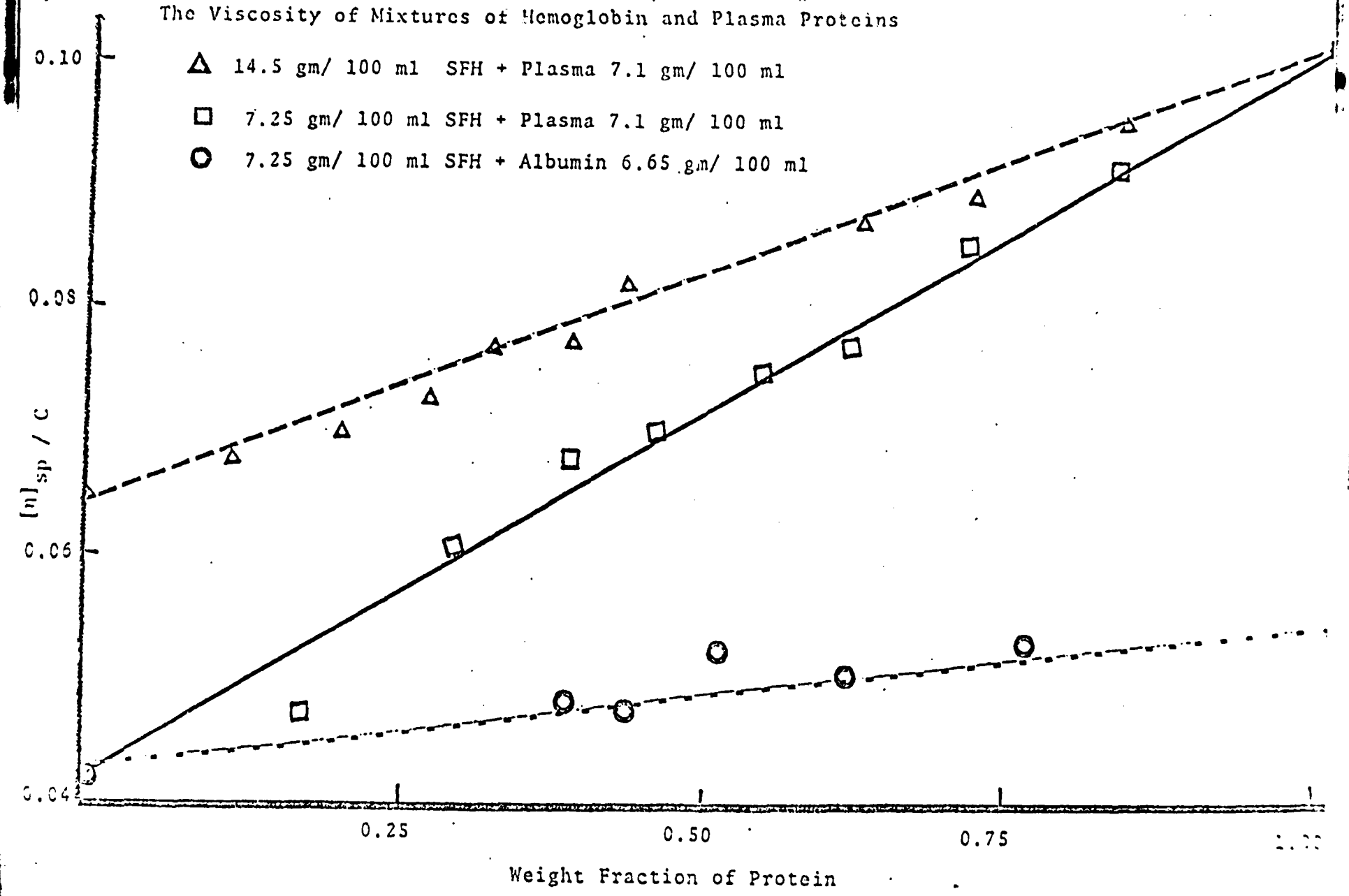


Figure 1:

The Relative Viscosity of Hemoglobin and Albumin Solutions

FIGURE 2 :

The Viscosity of Mixtures of Hemoglobin and Plasma Proteins



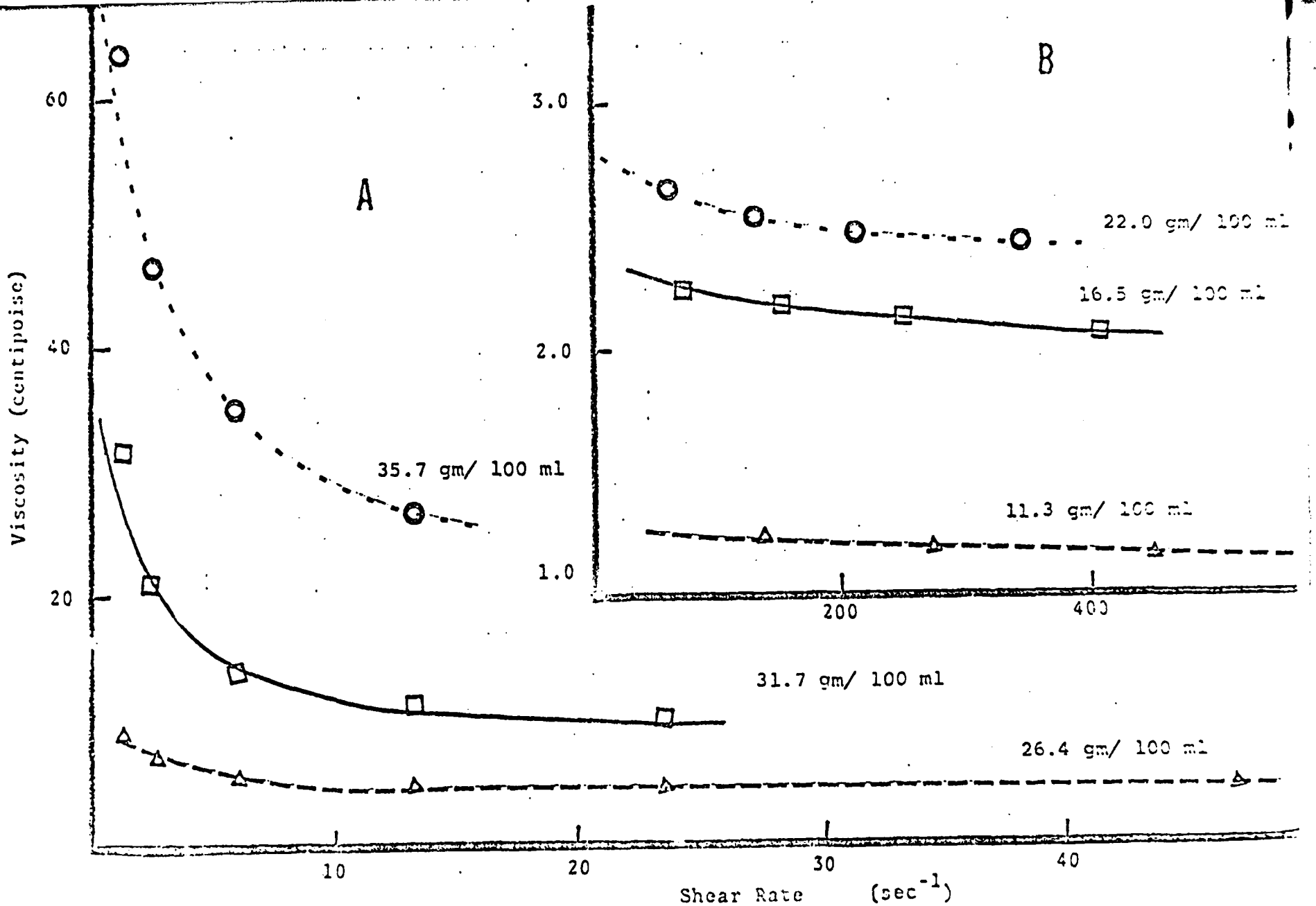


FIG 3: The Effect of Shear Rate on the Viscosity of Hemoglobin Solutions

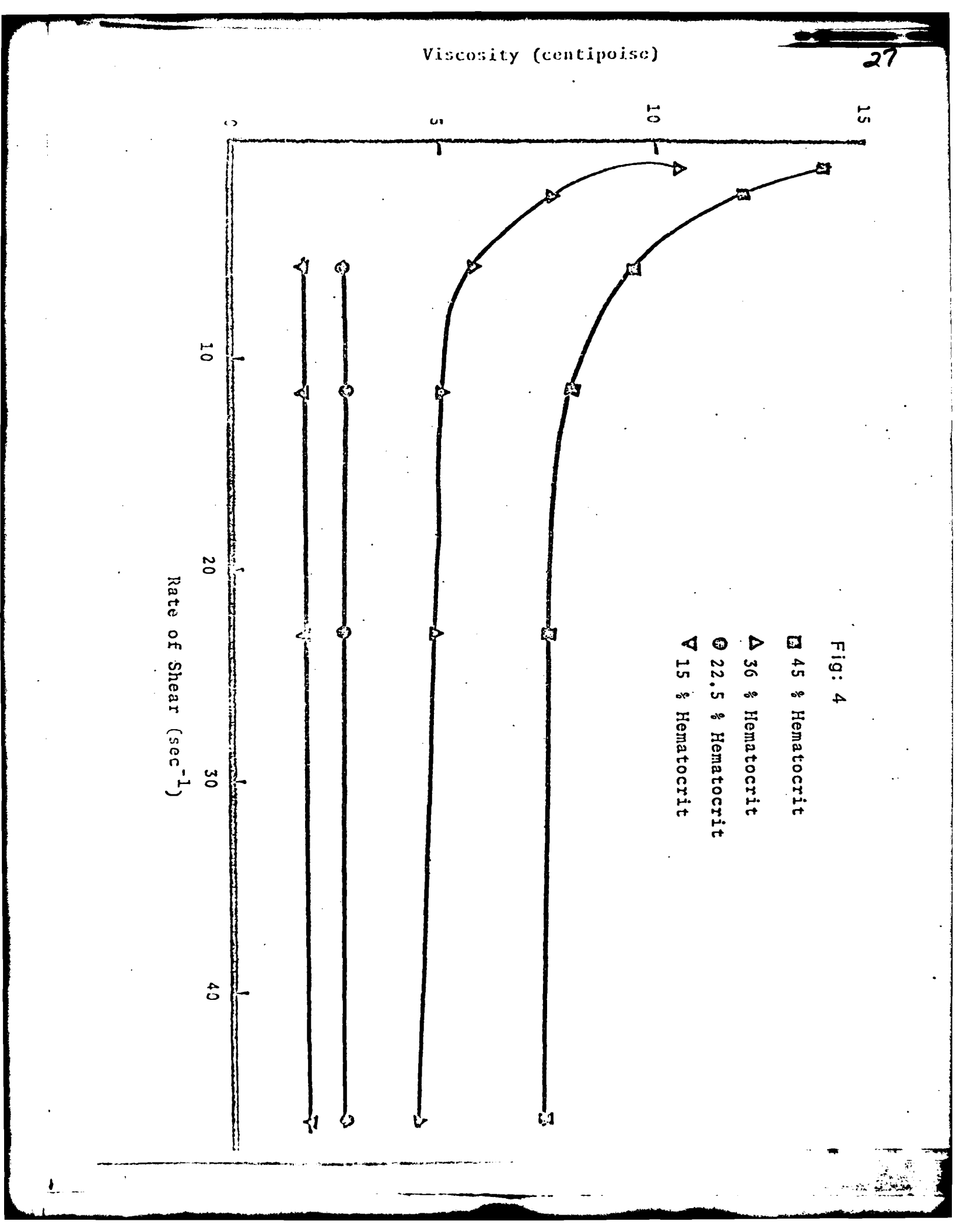

Viscosity (centipoise)
15
10
5
0
10
20
30
40
Rate of Shear (sec^{-1})
45 % Hematocrit
36 % Hematocrit
22.5 % Hematocrit
15 % Hematocrit

Fig: 4

Fig: 5

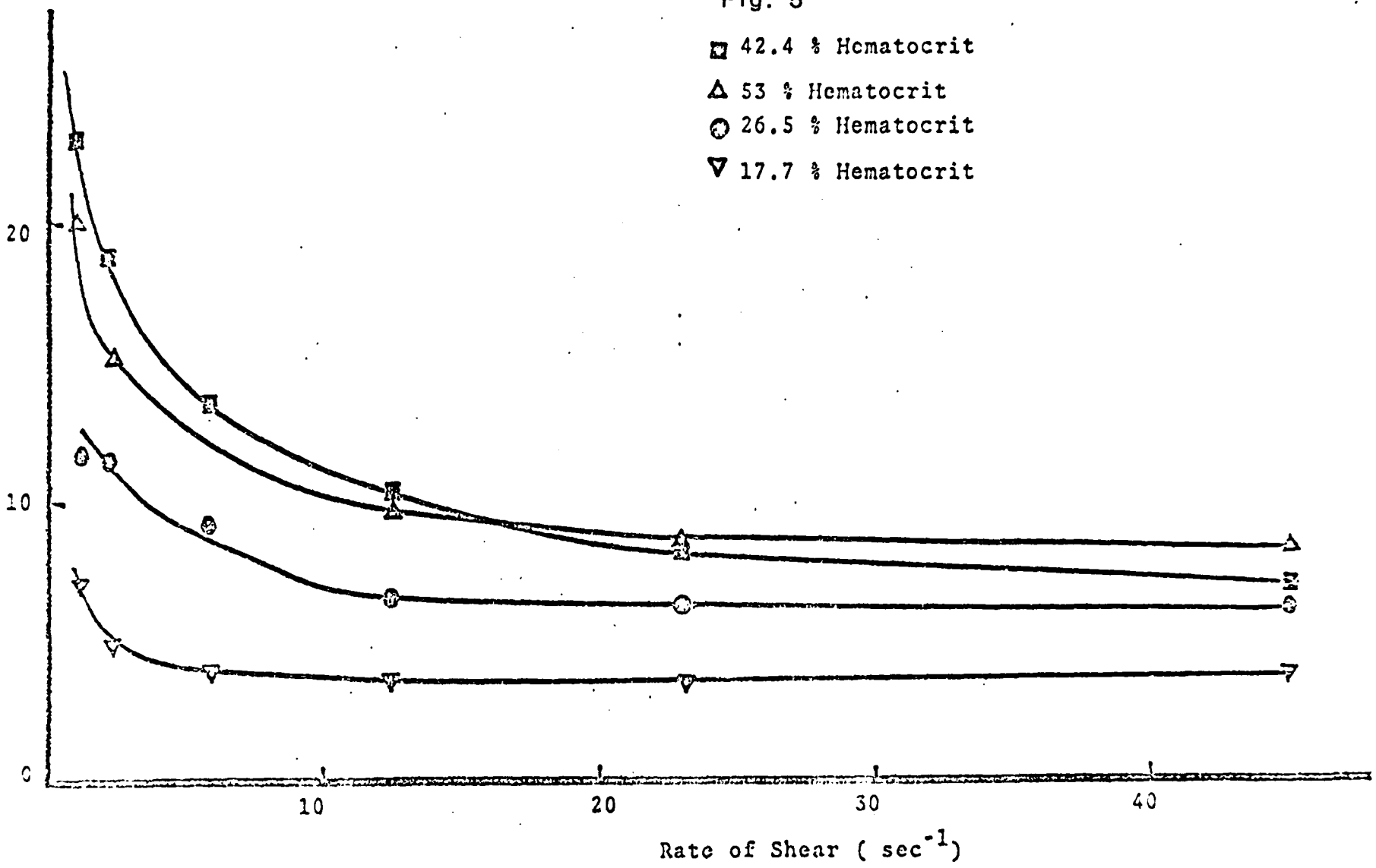

42.4 % Hematocrit
53 % Hematocrit
26.5 % Hematocrit
17.7 % Hematocrit
20
10
0
10
20
30
40
Rate of Shear (sec-1)

FIG. 6 : The Viscosity and Apparent Oxygen Transport in 6.1% SFH

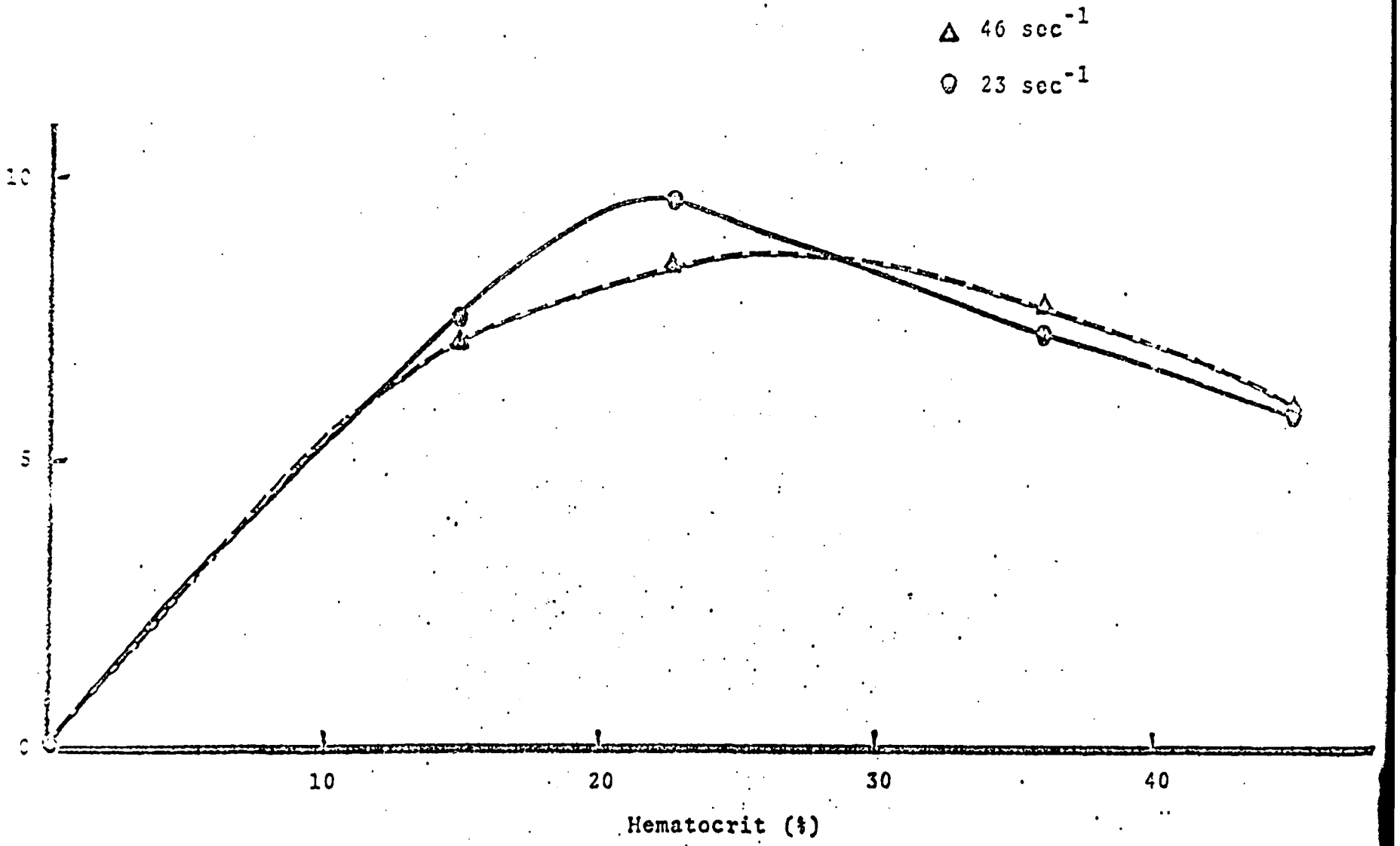


Fig: 7

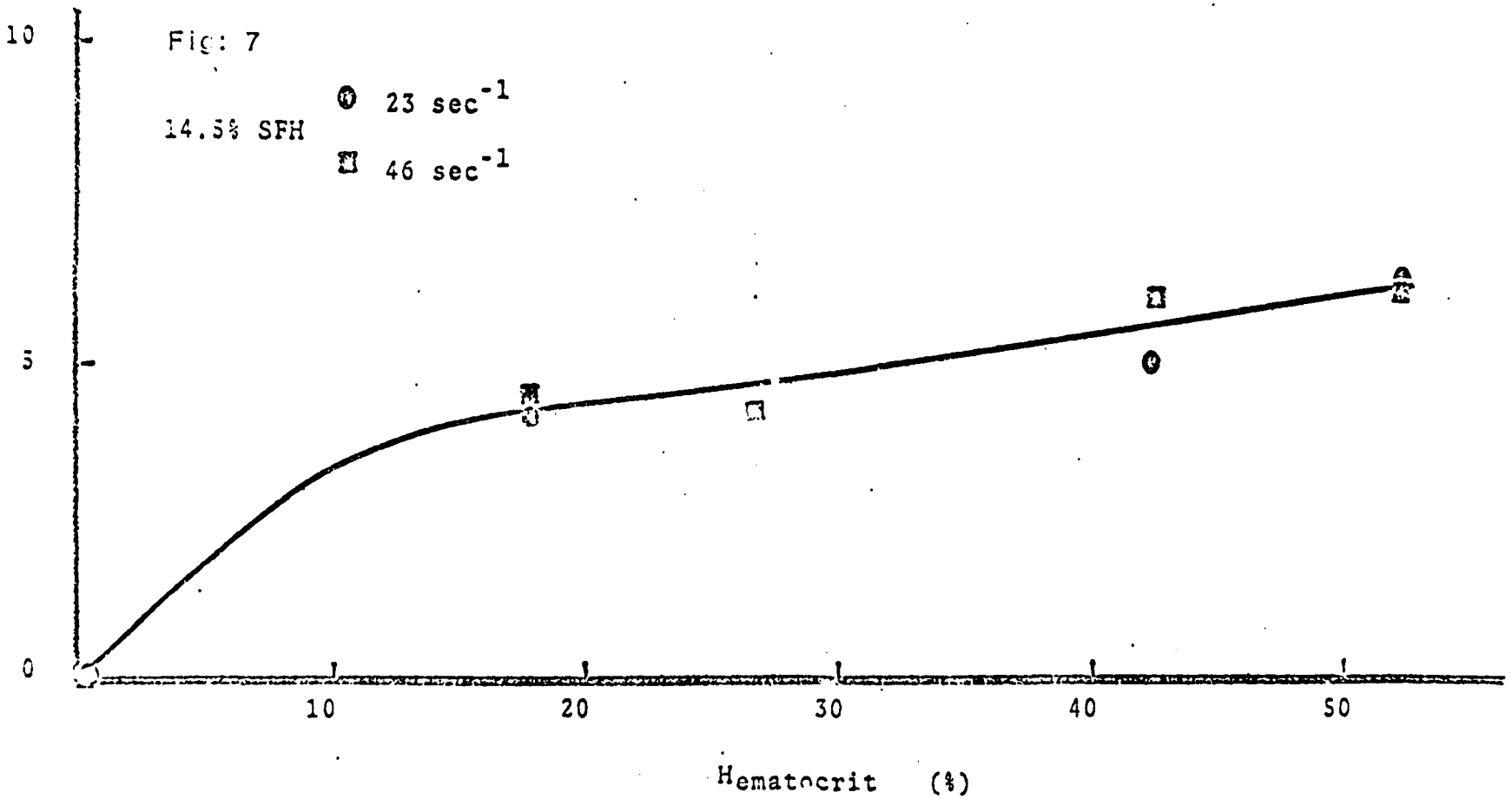
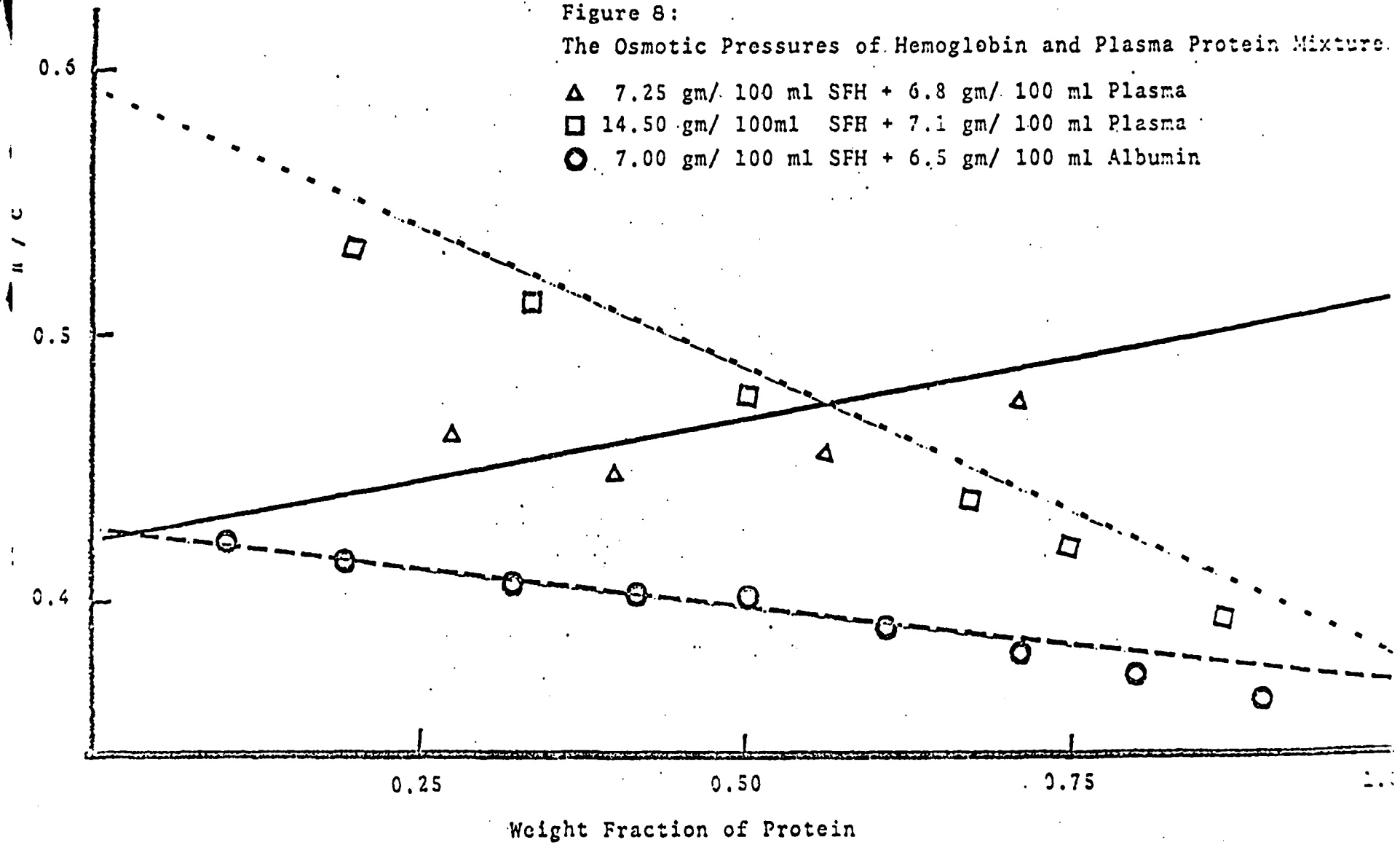


Figure 8:
The Osmotic Pressures of Hemoglobin and Plasma Protein Mixture.

△ 7.25 gm/ 100 ml SFH + 6.8 gm/ 100 ml Plasma
□ 14.50 gm/ 100ml SFH + 7.1 gm/ 100 ml Plasma
○ 7.00 gm/ 100 ml SFH + 6.5 gm/ 100 ml Albumin



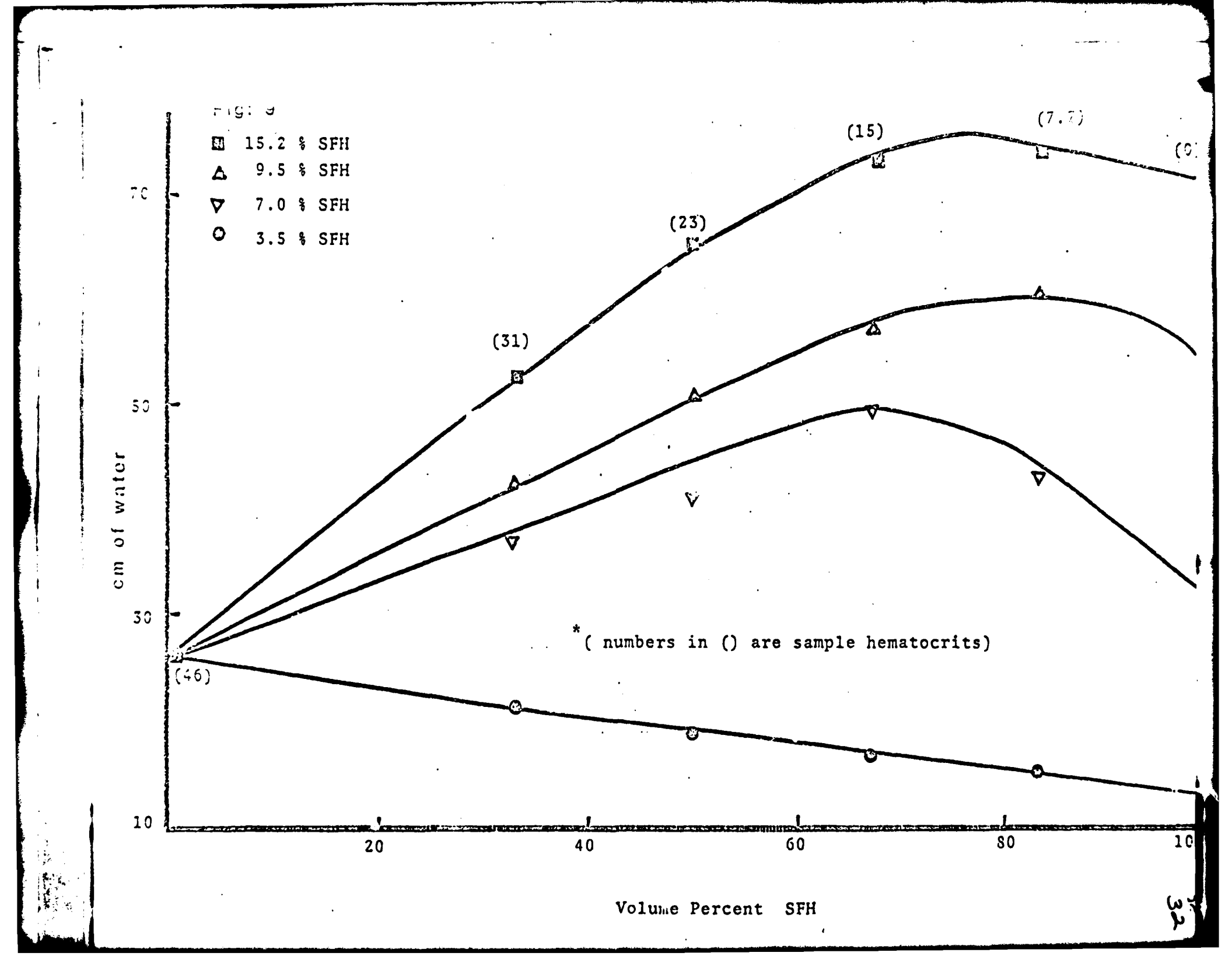


Fig. 9
15.2 % SFH
9.5 % SFH
7.0 % SFH
3.5 % SFH
(46)
(31)
(23)
(15)
(7.7)
(0)
*(numbers in () are sample hematocrits)
cm of water
70
50
30
10
20
40
60
80
10
Volume Percent SFH

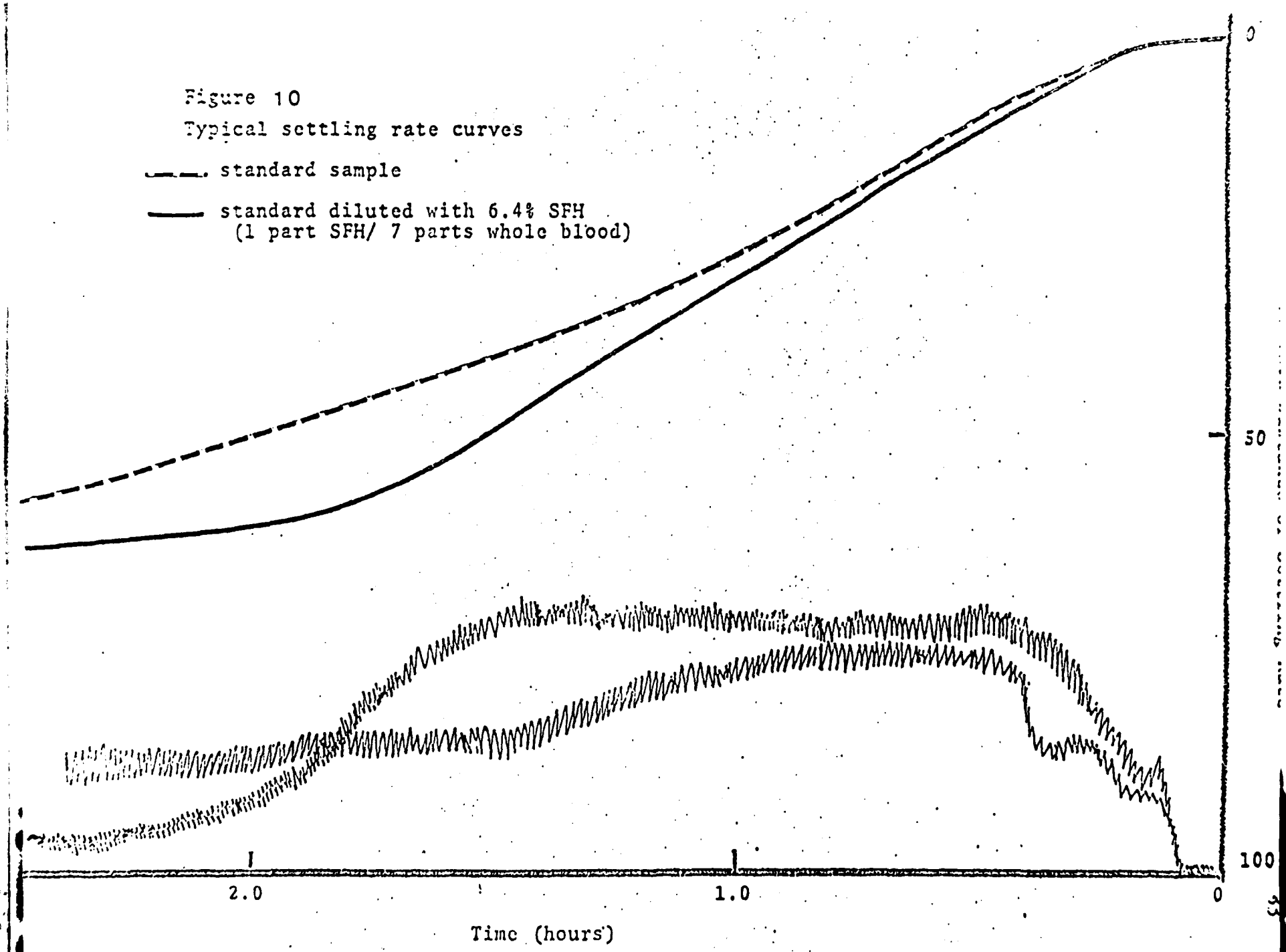


Figure 10

Typical settling rate curves

standard sample

standard diluted with 6.4% SFH
(1 part SFH/ 7 parts whole blood)

Fig: 11

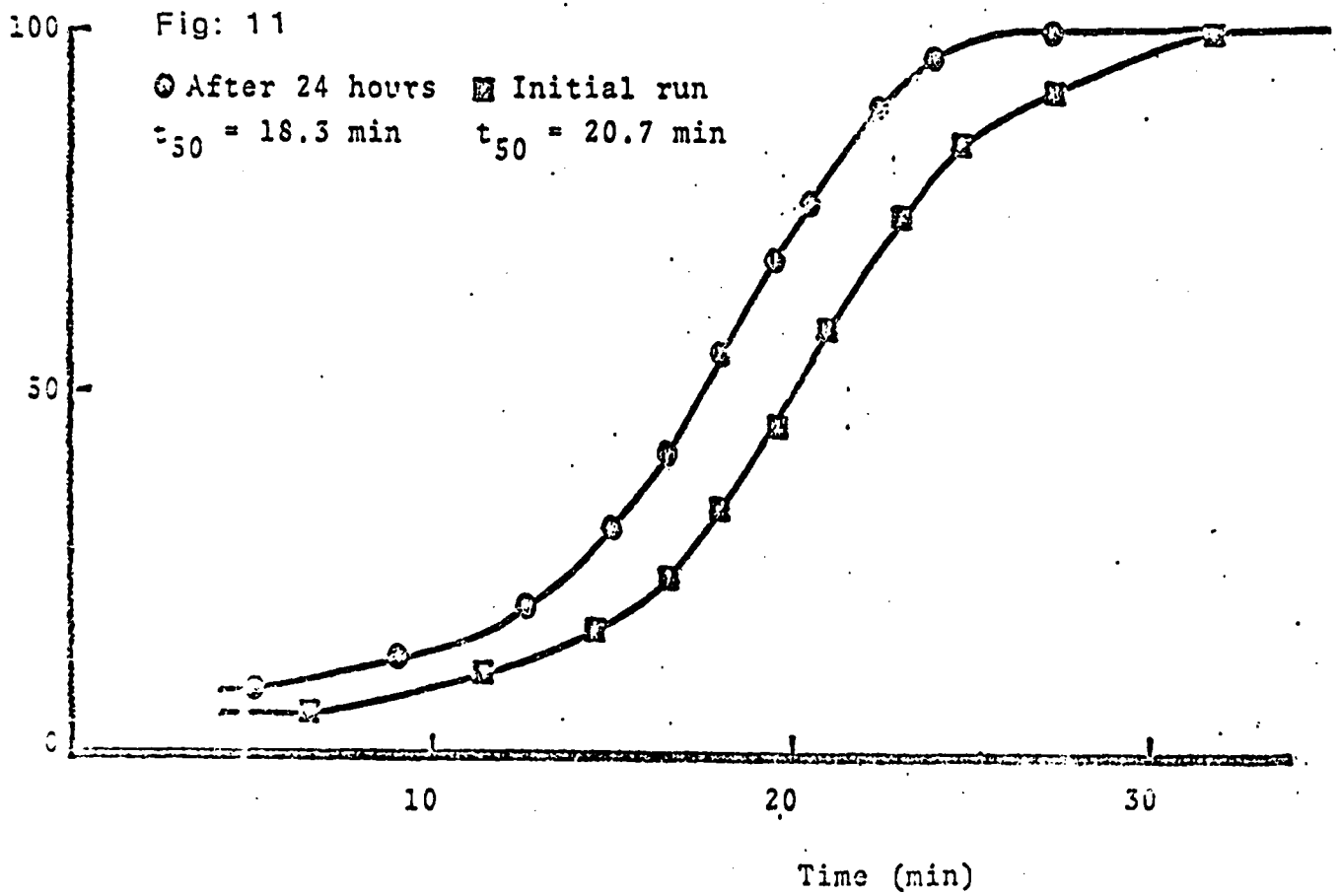
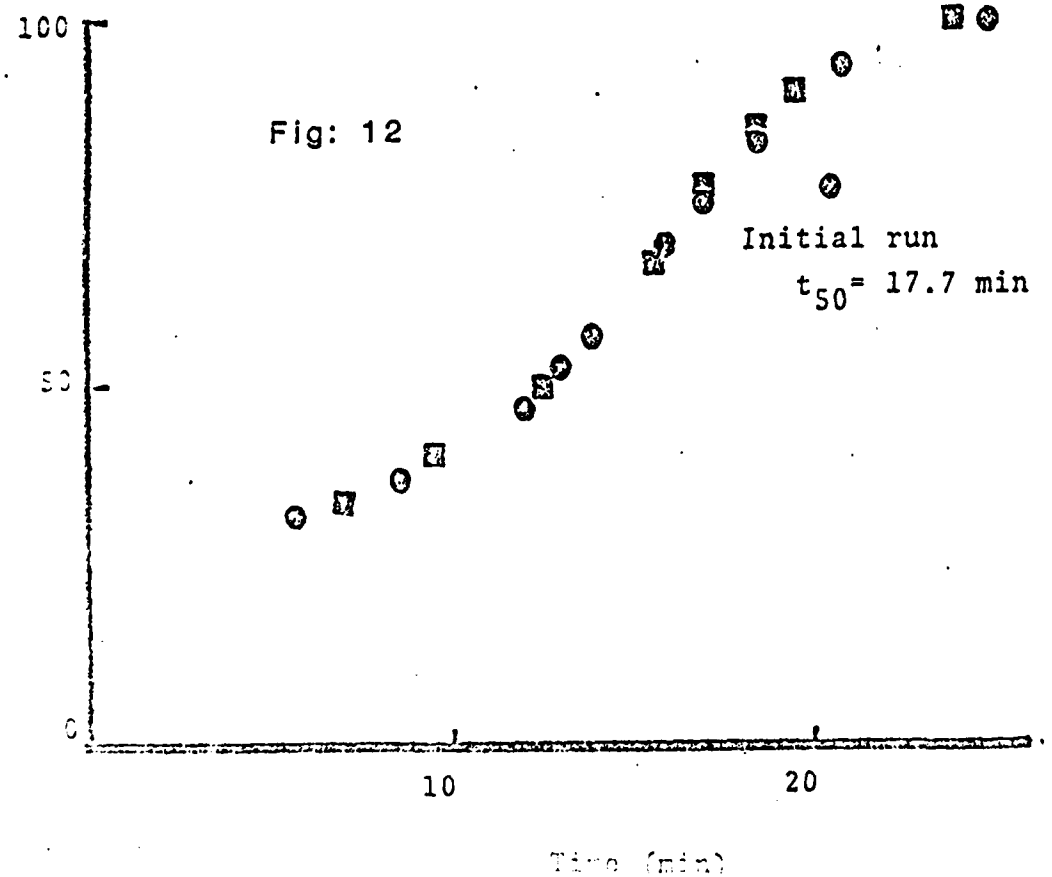

After 24 hours
t_{50} = 18.3 min
Initial run
t_{50} = 20.7 min
100
50
0
10
20
30
Time (min)

Fig: 12



Initial run t_{50} = 17.7 min

After 24 hours t_{50} = 18.2 min

FIG: 13

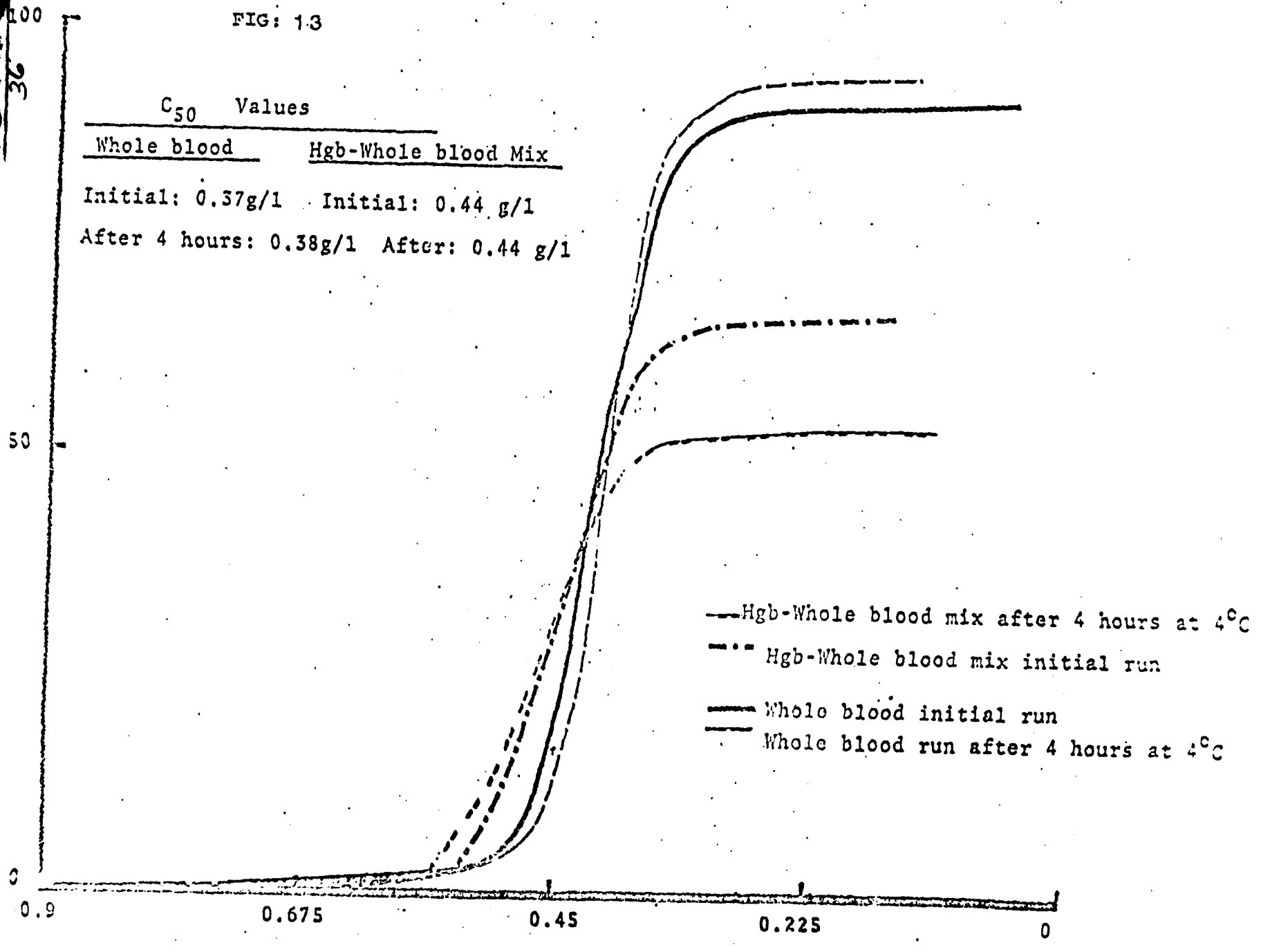

100
50
0
0.9
0.675
0.45
0.225
0
C_{50} Values
Whole blood
Hgb-Whole blood Mix
Initial: 0.37g/1
Initial: 0.44 g/1
After 4 hours: 0.38g/1
After: 0.44 g/1
Hgb-Whole blood mix after 4 hours at 4°C
Hgb-Whole blood mix initial run
Whole blood initial run
Whole blood run after 4 hours at 4°C

FIG: 14

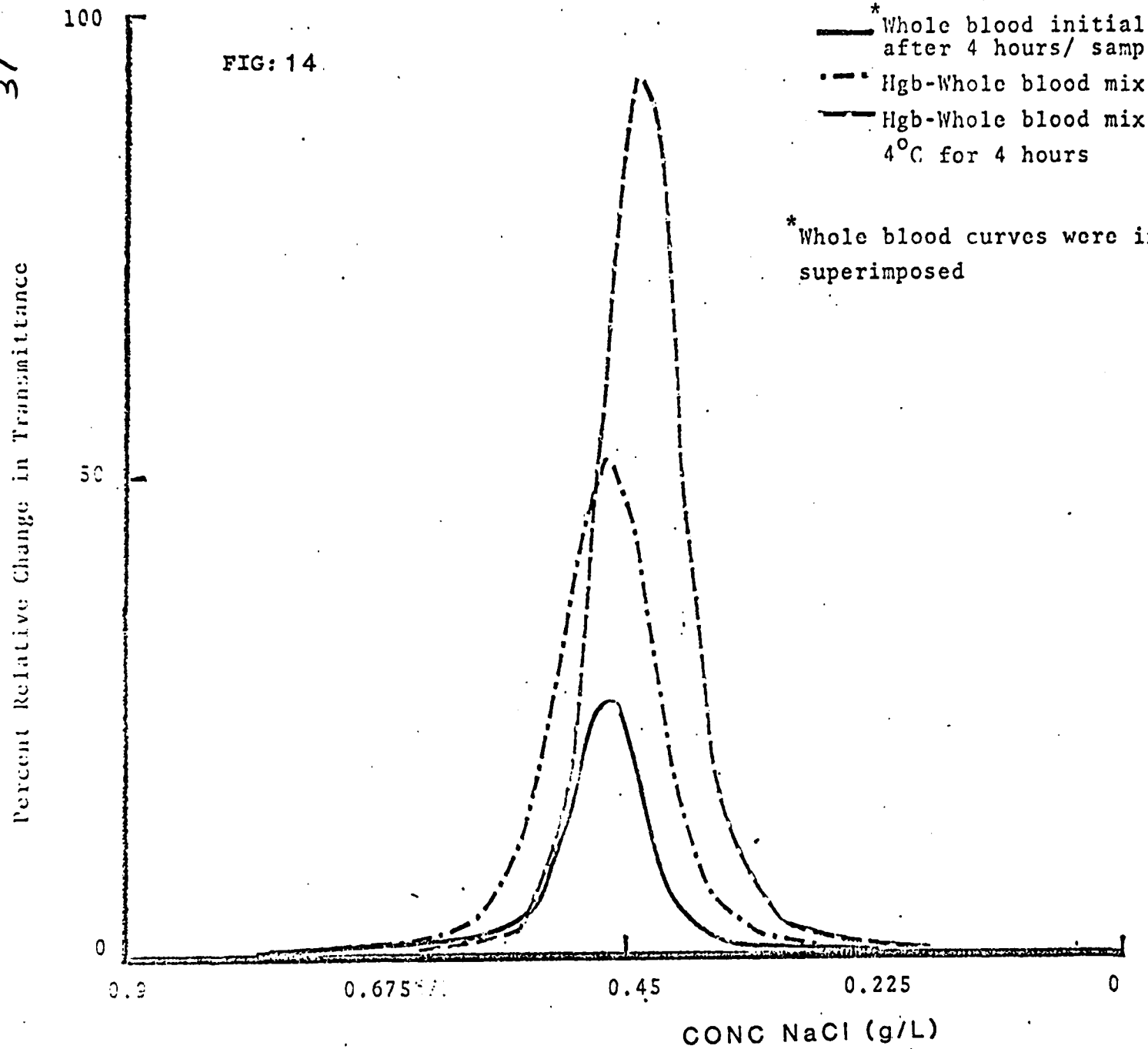

100
50
0
Percent Relative Change in Transmittance
0.9
0.675
0.45
0.225
0
CONC NaCl (g/L)
*Whole blood initial run and repeat after 4 hours/ samples at 4°C before run
Hgb-Whole blood mix (50/50) initial run
Hgb-Whole blood mix after storage at 4°C for 4 hours
*Whole blood curves were indistinguishly superimposed

FIG: 15

C_{50} Values

Whole blood	Hgb-Whole blood Mix
Initial: 0.46g/1	Initial: 0.47 g/1
After 4 hours: 0.45g/1	After: 0.46 g/1

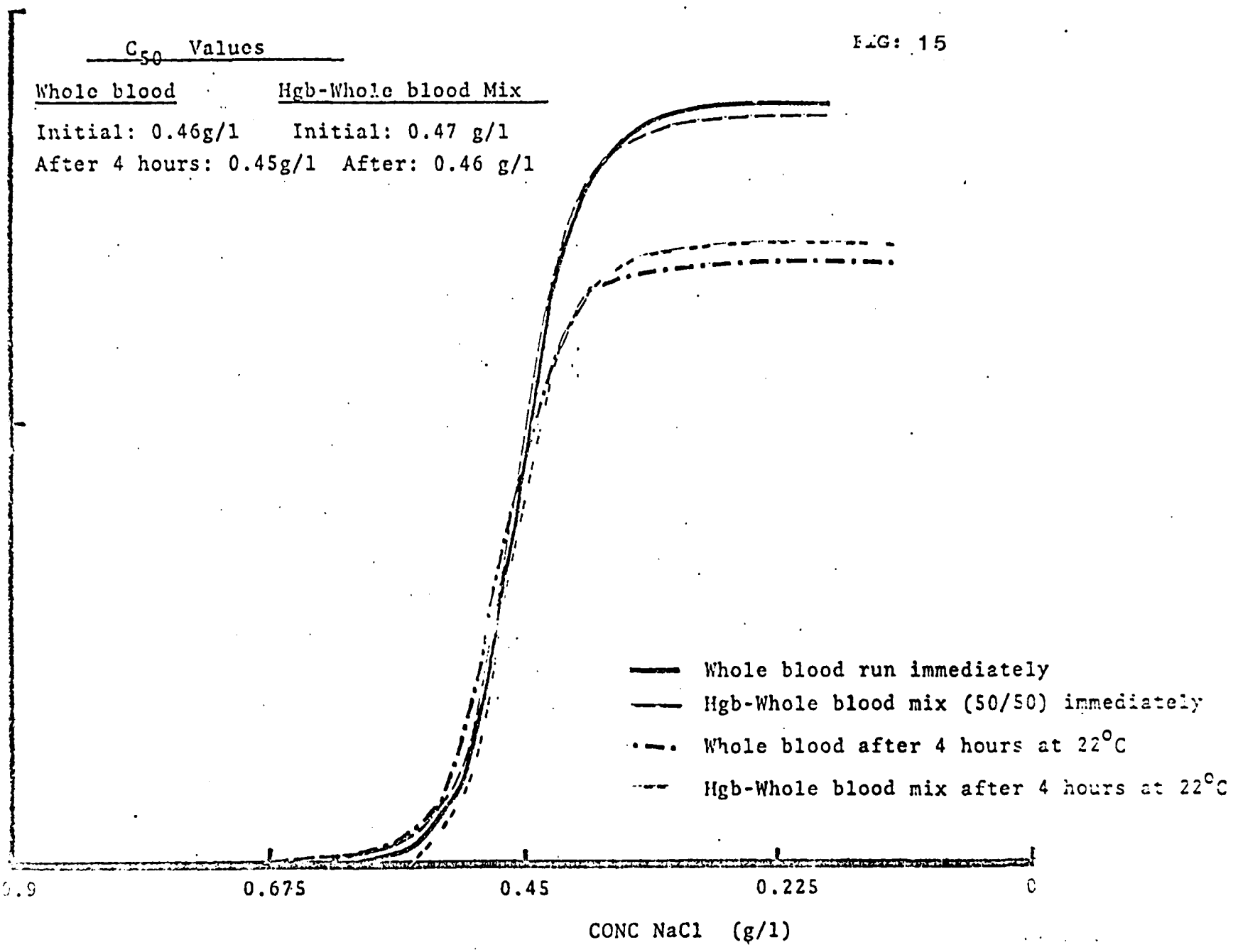


FIG: 16

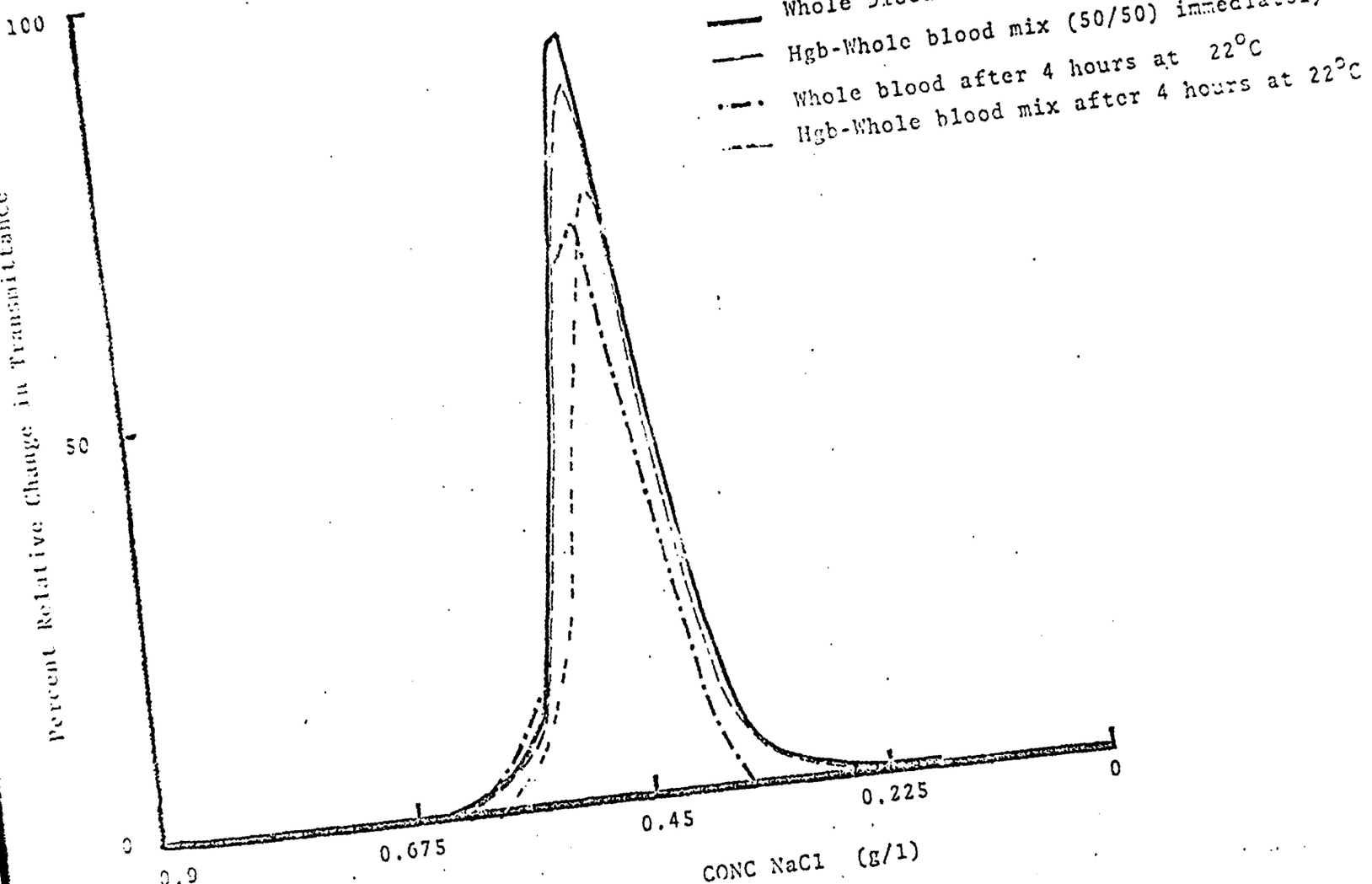
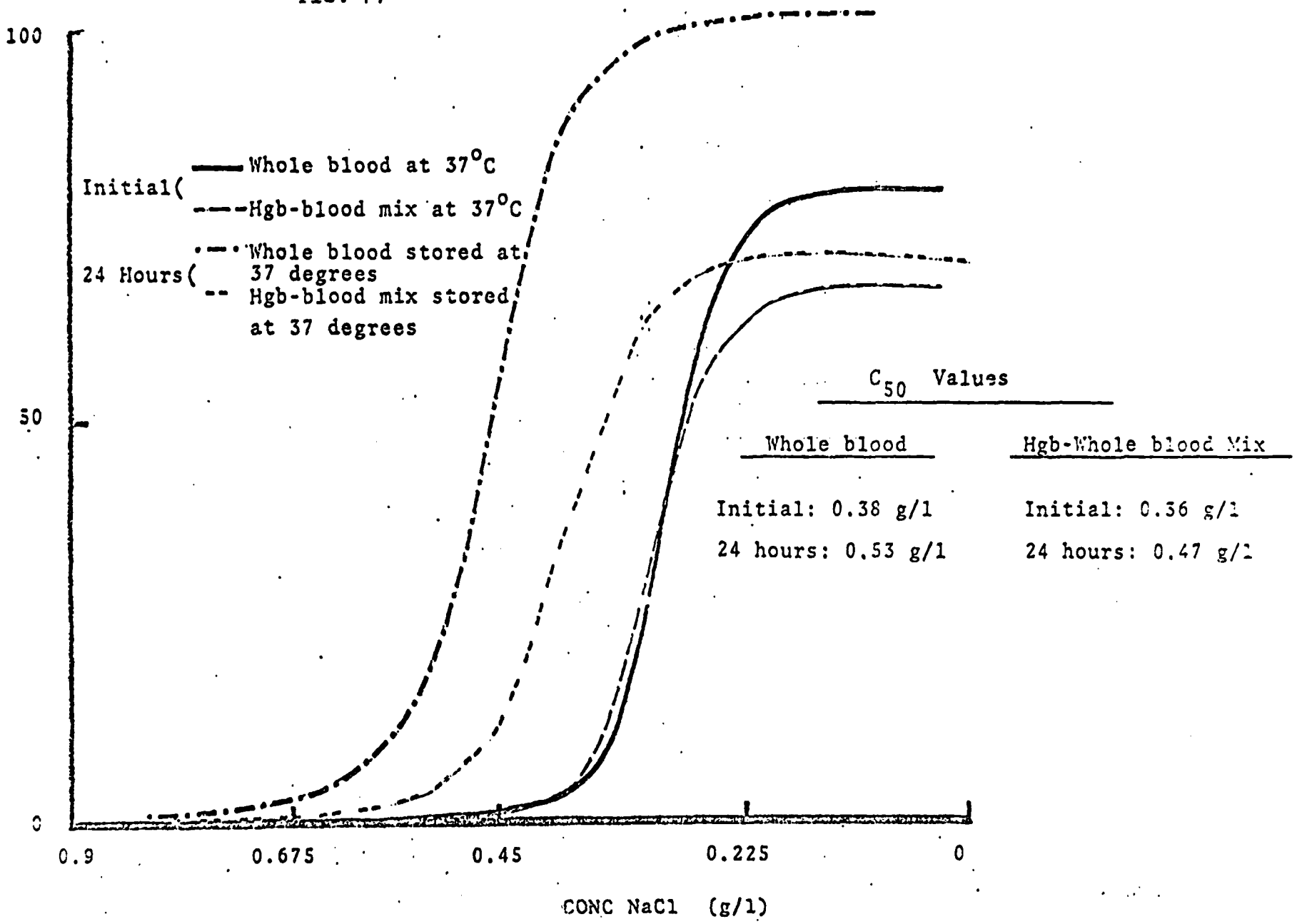

Whole blood run immediately
Hgb-Whole blood mix (50/50) immediately
Whole blood after 4 hours at 22°C
Hgb-Whole blood mix after 4 hours at 22°C
Percent Relative Change in Transmittance
100
50
0
0.9
0.675
0.45
0.225
0
CONC NaCl (g/l)

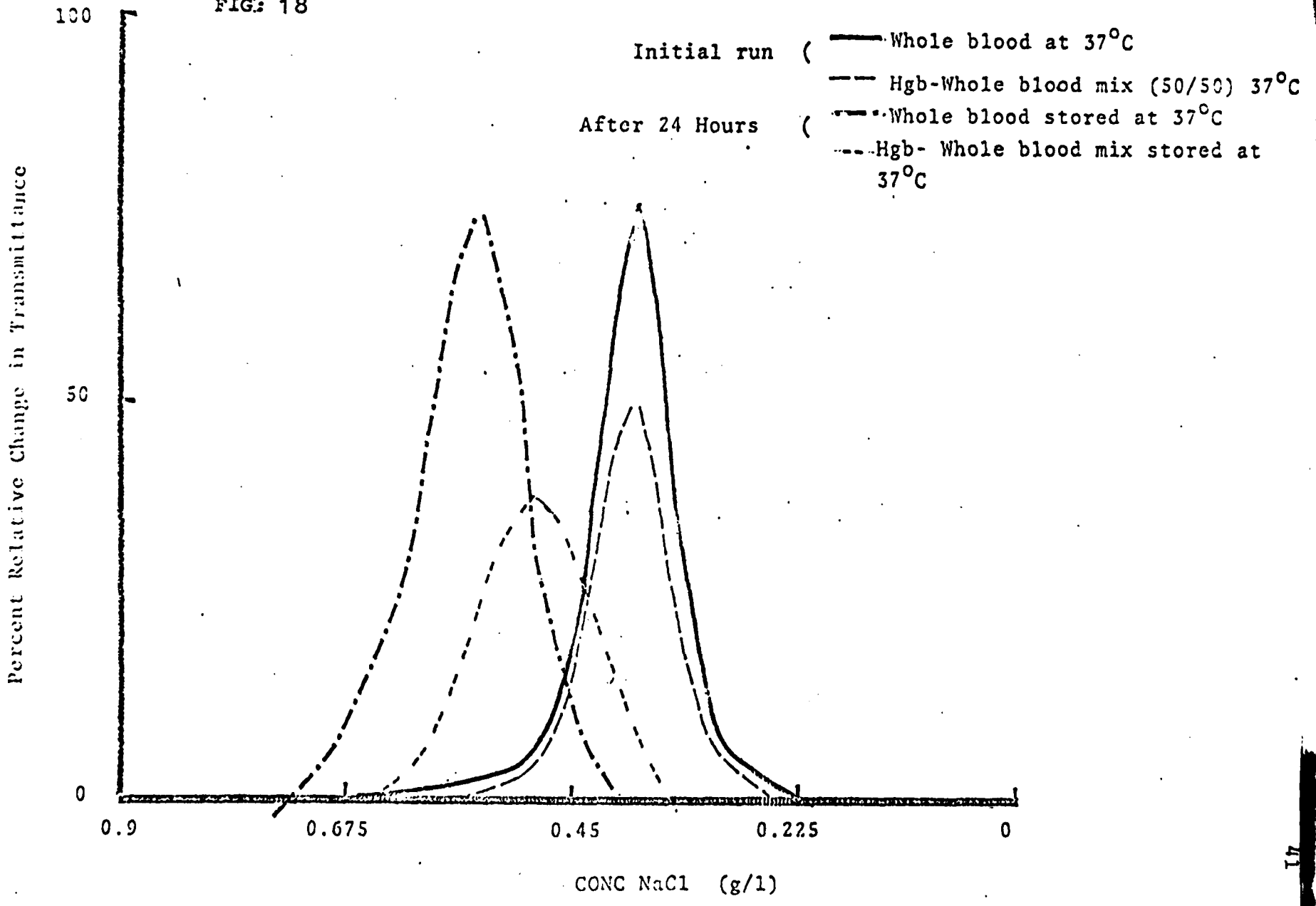
FIG: 17

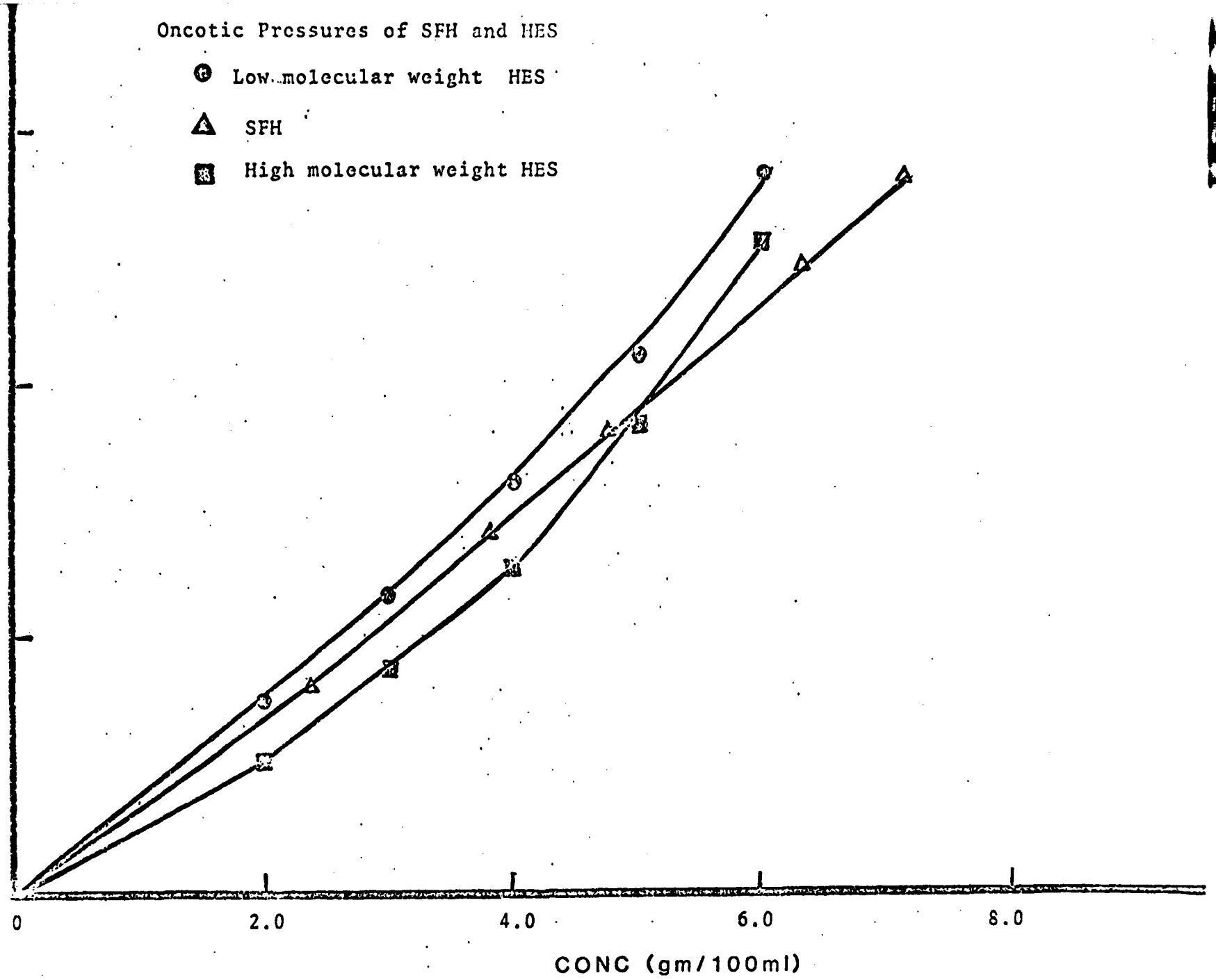


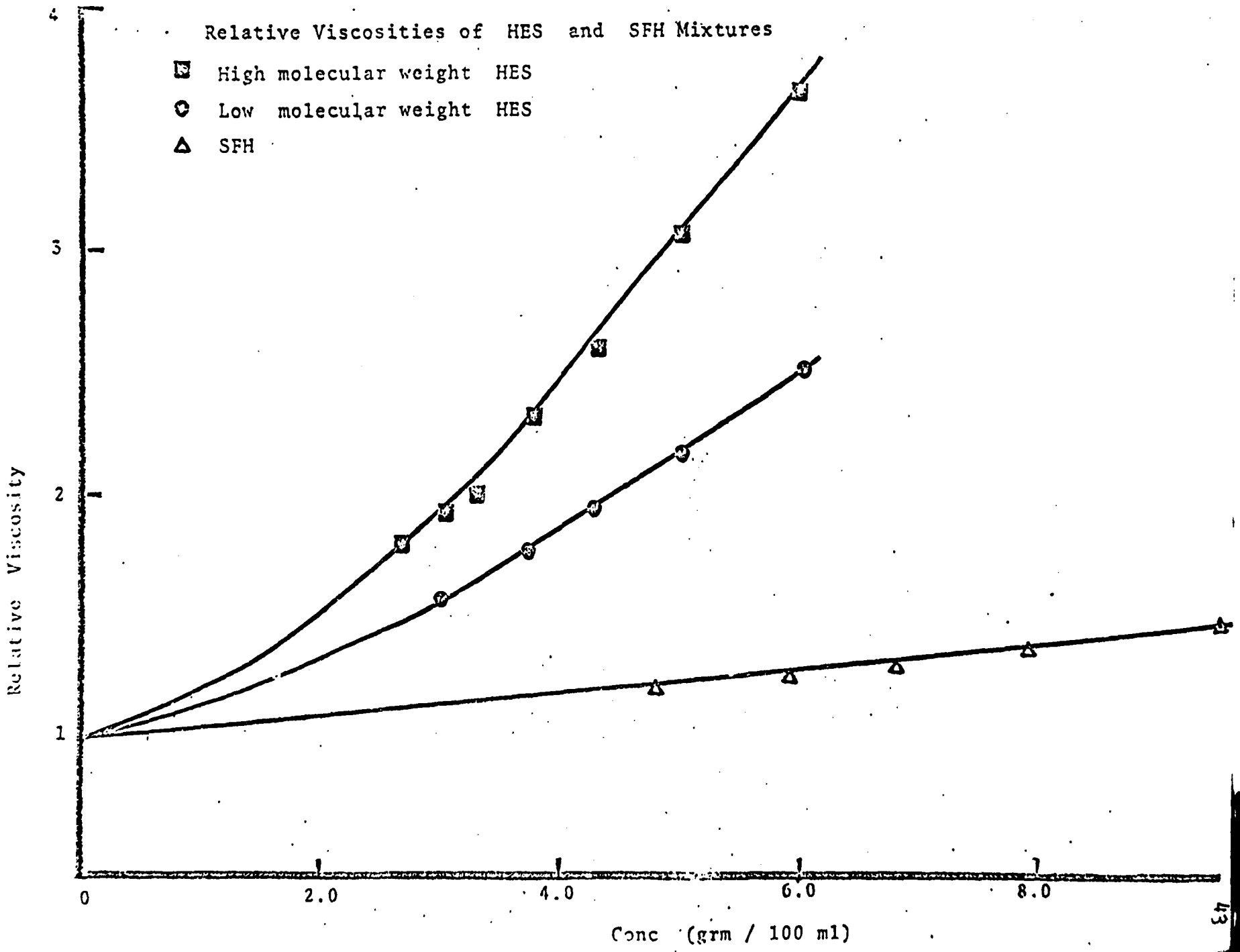
C_{50} Values

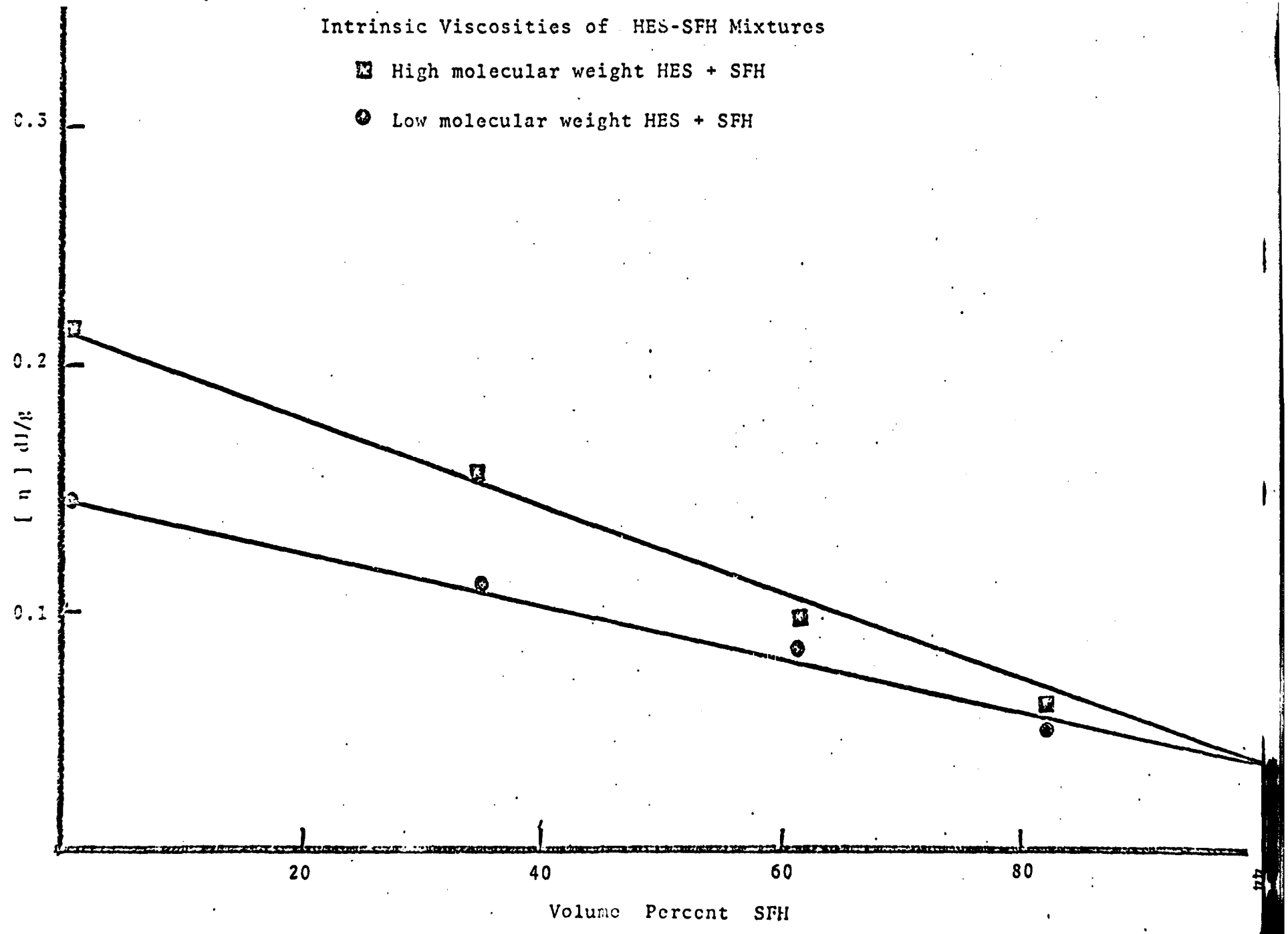
Whole blood	Hgb-Whole blood Mix
Initial: 0.38 g/l	Initial: 0.36 g/l
24 hours: 0.53 g/l	24 hours: 0.47 g/l

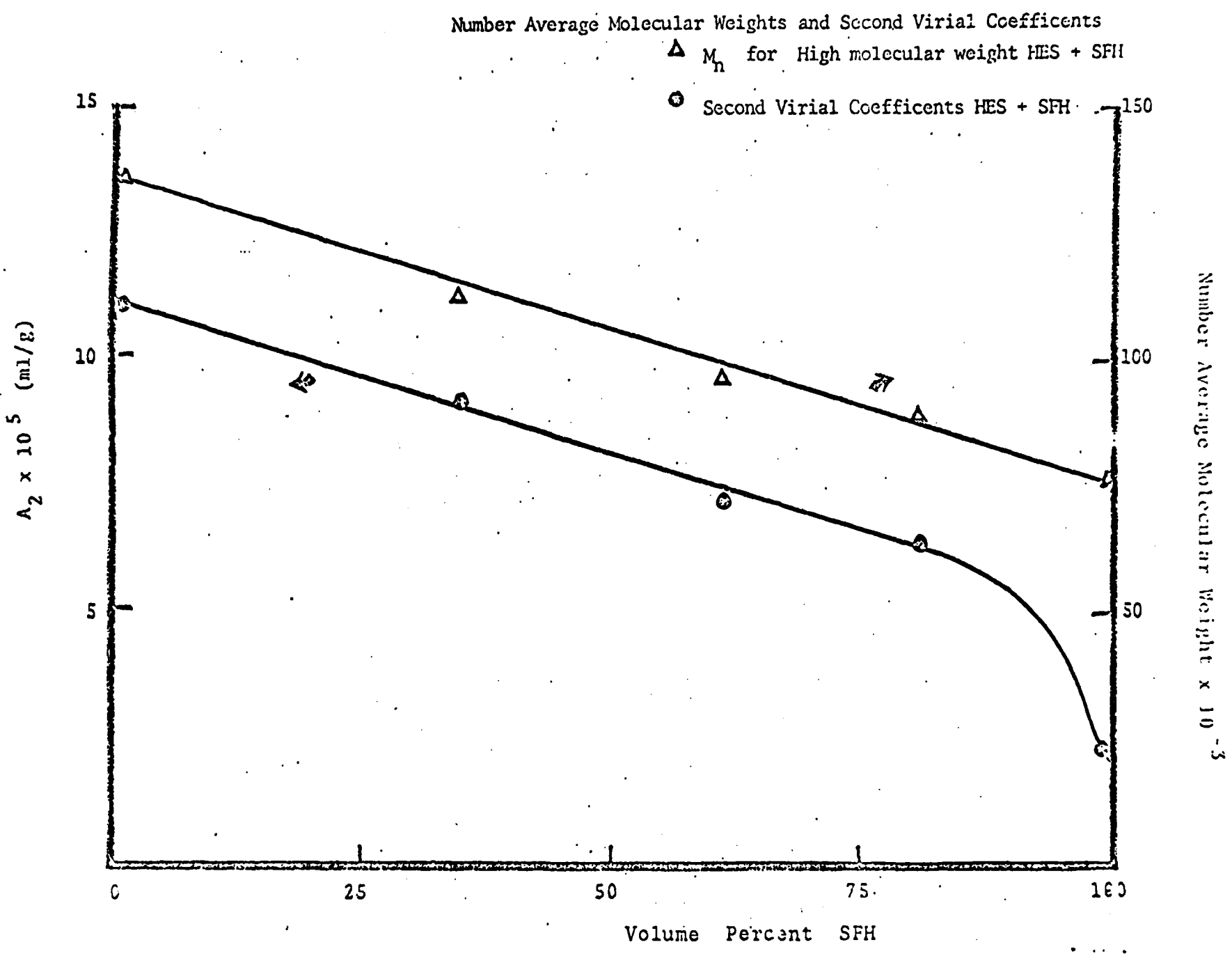
FIG. 18

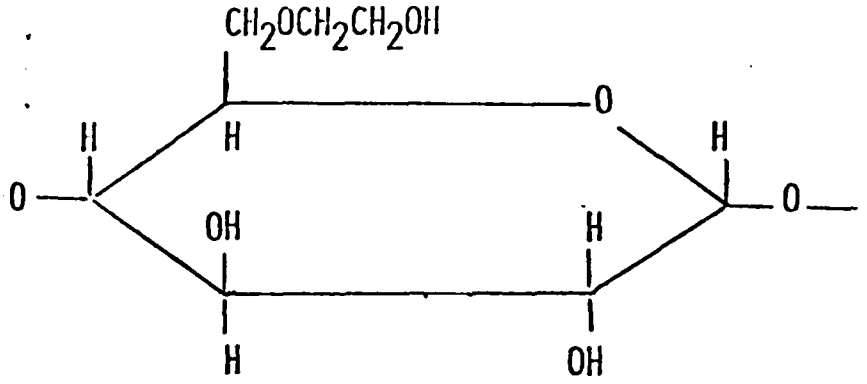

100
50
0
Percent Relative Change in Transmittance
Initial run (
Whole blood at 37°C
Hgb-Whole blood mix (50/50) 37°C
After 24 Hours (
Whole blood stored at 37°C
Hgb- Whole blood mix stored at 37°C
0.9
0.675
0.45
0.225
0
CONC NaCl (g/l)


Oncotic Pressures of SFH and HES
Low molecular weight HES
SFH
High molecular weight HES
30
20
10
h (cm of H_2O)
0
2.0
4.0
6.0
8.0
CONC (gm/100ml)


Relative Viscosities of HES and SFH Mixtures
High molecular weight HES
Low molecular weight HES
SFH
Relative Viscosity
4
3
2
1
0
2.0
4.0
6.0
8.0
Conc (grm / 100 ml)


Intrinsic Viscosities of HES-SFH Mixtures
High molecular weight HES + SFH
Low molecular weight HES + SFH
0.3
0.2
0.1
[η] dl/g
20
40
60
80
Volume Percent SFH


Number Average Molecular Weights and Second Virial Coefficents
Δ M_n for High molecular weight HES + SFH
Second Virial Coefficents HES + SFH
15
10
5
150
100
50
$A_2 \times 10^5$ (ml/g)
Number Average Molecular Weight x 10^{-3}
0
25
50
75
100
Volume Percent SFH



STRUCTURE OF A LINEAR UNIT OF HYDROXYETHYLSTARCH

H E S

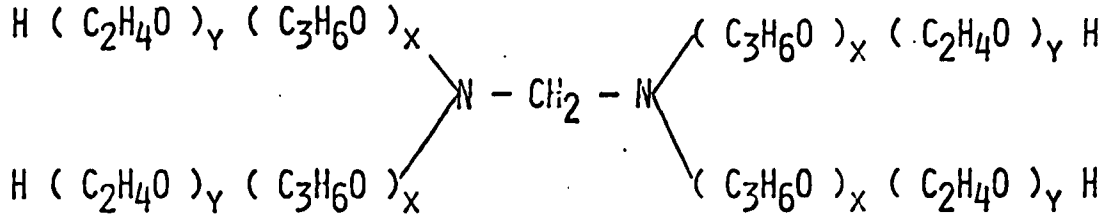
↓ CYANOGEN BROMIDE

↓ ETHYLENE DIAMINE

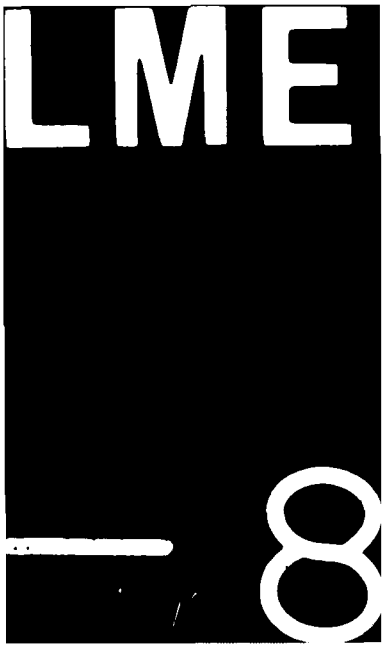
↓ GLUTARALDEHYDE

↓ HEMOGLOBIN

H E S – H G B POLYMER



BASIC STRUCTURE OF A TETRONIC POLYOL


LME
8